GREENHOUSE TESTING OF SLASH PINE FOR RESISTANCE TO
FUSIFORM RUST

By

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TABLE OF CONTENTS

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This study was designed to develop predictions of field breeding values for
fusiform rust resistance from early testing under greenhouse conditions. The
experiment was conducted at the USDA Forest Service Resistance Screening Center
(RSC) near Asheville, N.C. Six-week-old seedlings from four groups of 25 slash
pine families plus six seedlots with different levels of resistance were inoculated
with fusiform rust fungus in three different testing periods using the concentrated
basidiospore suspension technique. Six months after inoculation, the presence (1)
or absence (0) of ten different fusiform rust symptoms, the number of adventitious
shoots present in the galled area, and the number of galls per seedling were
evaluated. Several other traits were constructed by combining individual traits.
Also, traits specifying gall characteristics were evaluated on a single gall and on all
galls present on a given seedling. A total of 22 different traits were evaluated.

The results indicated that most traits ranked slash pine families consistently
across the different testing periods, but trait incidence levels varied from test to test;

therefore, to compare families tested in different tests it will be imperative to adjust the family means for the scale effects of the test in which they were tested. Also, transformation of the binomial traits, especially use of logistic transformation, reduced family by test interaction and should be considered as part of the analysis of RSC data on binomial traits.

Genetic parameters were estimated for the 16 most promising traits for predicting field breeding values. For 11 of the 16 traits, the estimated genetic parameters were reliable, had minor differences among the four groups of families, and had relatively high family heritability. Averaged over the four groups, for all traits the maximum family heritability was 0.90, and the minimum was 0.58, and 11 traits had heritability greater than 0.78.

Finally, the application of selection index theory to the greenhouse tests resulted in more efficient and easily interpreted predictions of parental breeding values for rust resistance than the index currently used by the RSC. Two indices were adopted, one with five traits for use with unimproved populations, and another with three traits for use with populations improved for fusiform rust. Selection based on breeding values predicted by the selected indices was less efficient than selection based on a single field progeny test of average quality. Nonetheless, the differences in selection efficiency were not large enough to rule out greenhouse testing as an alternative or additional testing technique for fusiform rust. It is recommend that the breeding program for slash pine be based on a combination of early testing in the greenhouse and short-term field tests designed specifically to evaluate rust resistance.

INTRODUCTION

Fusiform rust, caused by the fungus *Cronartium quercuum* (Berk) Miyabe ex Shirai f. sp. *fusiforme,* is an economically important disease of slash pine (*Pinus elliotii* var. *elliotii* Englem.) and loblolly pine (*Pinus taeda* L.) in the southern U.S., with estimated losses up to 35 million dollars annually (Anderson et al., 1986). Yield loss is associated with mortality of stem-galled trees and significant direct and indirect losses due to growth reduction, reduced marketability of the wood products, increased management costs, and sometimes complete loss of plantations (Holley and Veal, 1977; Schmidt et al., 1986). Although this disease is controlled in nurseries through proper fungicide application, use of genetically-resistant planting stock is the primary and most economically-feasible method of managing fusiform rust in pine plantations. Substantial genetic variation in resistance under field conditions exists within both species (Barber et al., 1957; Arnold and Goddard, 1965; Jewell and Mallet, 1967; Dinus, 1971; Schmidt and Goddard, 1971; Goddard et al., 1975) and considerable improvement is possible by phenotypic selection of rust-free trees in stands of high rust incidence (Goddard et al. 1977; Hodge et al., 1989).

Over the past three decades, genetic improvement for fusiform rust resistance in slash pine has been one of the major objectives of the Cooperative Forest Genetics Research Program (CFGRP) at the University of Florida. The CFGRP directs the breeding program of slash pine for 15 cooperating companies and agencies. These cooperators breed genetically-improved slash pine for use on over 6 million hectares of timberlands in areas with a very wide range of fusiform rust

1

hazard. The program began in the late 50s with cooperators searching their natural forests and plantations of slash pine, looking for phenotypically superior trees. Altogether, the CFGRP selected over 2,000 trees (called first-generation selections). These selections came from stands in Florida, Georgia, Alabama, and South Carolina, and are assumed unrelated to one another.

In order to assess parental breeding values for rust resistance, open-pollinated progenies from selected parents were evaluated by both regular progeny tests and high hazard progeny tests. The first method, regular progeny testing, was designed to assess both growth rate and rust resistance, and tests were planted over a range of sites without regard to rust hazard levels. Many of these field tests experienced very low rust incidence and were of limited use in differentiating resistant from susceptible families (Schmidt and Goddard, 1971). On the other hand, moderate to high incidence of disease in a given test increased precision for assessing rust resistance but reduced the precision for evaluation of other traits such as growth and wood properties (Hodge and White, 1986). Other shortcomings of the regular field tests are the length of time required for the test (i.e., 3 to 10 years) and the overall cost of each test. High hazard progeny tests were designed to assess only rust resistance and were planted in field areas exposed to high levels of rust inoculum. Measurements were collected at an intermediate age (5 years) after which time the tests were terminated. In this case, two series of field tests were needed, high hazard tests for rust and another series for growth evaluations. Based on data from both testing procedures, breeding values for rust resistance of all 2,245 slash pine first-generation parents were predicted using best linear predictions (BLP) (White and Hodge, 1988).

The CFGRP has now formulated its second-generation selected population for slash pine. This population has 886 genotypes: 404 are the best original first-generation selections; 304 are the best individuals phenotypically selected from the

better 2,700 full-sib families created by crossing better than average first-generation parents; and 178 are untested infusions (mass selections from natural populations) (White et al., 1989). An indirect testing procedure that quickly screened families for rust resistance, could conceivably reduce the time to obtain breeding value predictions. The predicted breeding values from such a procedure could be used to retain only the best parents in the breeding population before crossing; to eliminate some parents before field tests to reduce the size and costs of field tests; and to select only the best parents to be included in seed orchards to increase gain and reduce the establishment and maintenance costs of the orchards.

An indirect testing technique for fusiform rust resistance was first devised by Jewell (1960) and has been under constant improvement throughout the years (Miller, 1970; Powers et al., 1971; Laird and Phelps, 1975; Matthews et al., 1978; Anderson et al., 1983; Knighten et al., 1988). Once the system was found to be feasible for large scale testing, the Resistance Screening Center (RSC) was established by the U.S. Dept. of Agriculture, Forest Service at Asheville, NC, to operationally screen large numbers of pine families as a service for several organizations, researchers, and private industries (Anderson and Powers, 1985). The current screening technique is presented by Knighten et al. (1988). Using very young seedlings (8 months old) and controlled greenhouse conditions, the RSC seeks to predict the relative resistance of pine families to fusiform rust as it would be expressed in the field on older trees (3 to 10 years old). Several greenhouse traits have been identified, that when combined in a selection index, might allow precise and consistent prediction of field breeding values from RSC results (Walkinshaw et al., 1980; Hubbard, 1981). However, to use this technique efficiently in the slash pine breeding program, the quantitative genetic parameters of the greenhouse traits and their relationship to field resistance are needed to allow prediction of field performance from RSC data. Once the system has been developed it will be

possible to assess the efficiency of the greenhouse testing procedures relative to field tests.

This dissertation is divided into three major chapters. In the first chapter, we evaluated the effects of different greenhouse testing periods (i.e., season of inoculation) on expression of 22 fusiform rust symptoms on three slash pine seedlots of varying levels of field resistance. The objectives were 1) to evaluate how transformations of greenhouse data affected the potential usefulness of the different traits for discriminating among families of varying levels of rust resistance, and 2) to identify which traits are most stable across different testing periods and are more likely to be useful in a index to predict field breeding values. The second chapter deals with the estimation of genetic parameters (i.e., family variance, variance of family means, family heritability, and genetic correlation) for several greenhouse traits, and for four independent groups of 25 open-pollinated slash pine families each. Each group of families was screened at the RSC in three different testing periods. The goal was to identify traits that are promising for inclusion in a selection index. A promising trait is considered one that has low family x test interaction, high heritability, and stable family variance and variances of family means across tests. Finally, the third chapter deals with application of selection index theory to predict field fusiform rust breeding values from measurements of one or more greenhouse traits. The goal was 1) to determine which traits should be combined in a selection index to allow prediction of field breeding values for populations unimproved and improved for rust, 2) to assess the relative efficiency of the RSC test compared to field tests in determining resistance to fusiform rust in open-pollinated slash pine families, and 3) to explore when and how greenhouse tests at the RSC should be used in slash pine breeding program.

EVALUATION OF SEASON OF INOCULATION AND FUSIFORM RUST SYMPTOM EXPRESSION ON SLASH PINE SEEDLOTS WITH DIFFERENT FIELD LEVELS OF RUST RESISTANCE

Introduction

Traditionally, resistance to fusiform rust *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* has been evaluated by field tests. These field tests require a long time to complete and are costly. Attempts to find early selection methods ultimately led to the concentrated basidiospore technique (Jewell, 1960; Miller, 1970; Powers et al., 1971; Matthews and Rowan, 1972; and Laird and Phelps, 1975). This greenhouse system has been used at the Resistance Screening Center (RSC), established by the USDA Forest Service at Asheville, NC, to operationally screen large numbers of pine seedlings (Anderson and Powers, 1985).

Initially, a single greenhouse trait (percentage of galled seedlings) was used by the RSC to evaluate the relative field resistance of pine families. This trait correctly discriminated resistant from susceptible families, but not families which, under field conditions, had an intermediate level of rust resistance (Dinus, 1969). Later, Walkinshaw et al. (1980) suggested that better field predictions could be obtained from an index based on regression analysis with three greenhouse traits which explained 62% of the variation in field performance of the families tested. Hubbard (1981) re-evaluated Walkinshaw's data and recommended a new index to predict relative resistance, based on standardized family expression of three traits. Later, a fourth trait was added to this index (Knighten et al., 1988).

This previous trait selection and recommended index were developed using data from few field tests, few families and a single greenhouse test (i.e., season of inoculation). It is possible that uncontrolled natural variation of greenhouse conditions due to seasonal environmental variation affects the expression of some traits more than others. Also the effect of season on expression of some greenhouse traits might be different for families with different levels of resistance. Family by environment interaction has been demonstrated for fertilization (Rowan, 1977), inoculum source (Snow et al., 1976), and inoculum density (Matthews et al, 1978); thus, there is a likelihood of family by test (i.e., season of inoculation) interaction as well. For maximum usefulness of this greenhouse screening technique, a trait or combination of traits should allow for effective discrimination among families over a broad range of resistance levels and across all seasons of inoculation.

Most traits (symptoms) measured on inoculated seedlings in the greenhouse are scored as presence (1) or absence (0) and are therefore binary on an individual seedling basis. Thus, the assumptions of normality and homogeneity of variance required for conventional analysis of variance are usually lacking. In addition, the ability to discriminate and/or predict family resistance or susceptibility is greatly reduced when the level of incidence for a given trait falls below 30% or goes above 70%, the non-linear portion of the distribution (Lush et al., 1948). A conventional approach to this type of data is to use transformations. Among the most common transformations (i.e., arcsin, logistic, probit, log-log), the logistic and arcsin transformations appear promising (Fisher, 1954; Naylor, 1964; and Cox, 1970).

This study is the first of a series designed to develop predictions of field breeding values for rust resistance from greenhouse (RSC) measurements. The overall goals of this study were to evaluate how transformation of greenhouse data affected the potential usefulness of the different traits for discriminating among slash

pine families of varying levels of field rust resistance and to identify which traits are most stable across testing periods (i.e., season of inoculation) and are most likely to be useful in a predictive index. The specific objectives were as folows: 1) To determine the effect of season and week of inoculation on the expression of many symptom traits, 2) To evaluate the benefits of the logistic and arcsin transformations for each trait, 3) To determine which greenhouse traits efficiently discriminate among seedlots known to differ in field resistance to rust (susceptible, average and resistant seedlots), 4) To determine if the traits have a linear or quadratic relationship with the seedlot field levels of rust resistance, and 5) To determine if it is useful to measure more than one gall per seedling.

Materials and Methods

Each of the six seedlots was a mixture of seeds from 5 to 20 open-pollinated slash pine families from different seed orchards in the Florida Cooperative Forest Genetics Research Program (CFGRP). The seedlots were classified according to the average predicted field parental breeding values for rust resistance as susceptible, average, and resistant to fusiform rust, with two seedlots in each of the three classes (Table 1.1). Seedlots in the same classification were mixed from the same set of parents, and thus can be considered as replications. The parental breeding values used to establish the three levels of field rust resistance were predicted from information from at least four field tests using best linear prediction methodology (White et al., 1986; and White and Hodge, 1987).

The experiment was conducted at the RSC using their standard concentrated basidiospore suspension technique (Anderson et al., 1983; and Knighten et al., 1988). In brief the procedures were seed stratification; seed sowing in 10 x 15 x 5 cm germination trays in the greenhouse; transplanting germinants into Ray Leach

Table 1.1. Composition, classification and average predicted field breeding values for fusiform rust resistance for the six slash pine seedlots used in the greenhouse screening test.

Seedlot	Classification	No. of Parents	Breeding Value (%)[a] Mean ± Standard Dev.
1 and 2	susceptible	9	86 ± 12
3 and 4	average	20	50 ± 16
5 and 6	resistant	5	-10 ± 20

[a] The parental breeding values were obtained from the CFGRP. Each breeding value predicts the average percentage of rust infection in a field environment where unimproved checks (i.e., average material) would incur 50% infection. The resistant seedlots are predicted to have -10% because the prediction methodology is linear and places no bounds on the prediction.

tubes (three weeks after sowing); seedling inoculation (five weeks after transplanting) with a concentrated basidiospore suspension (20,000 spores per ml) composited from spores collected in five slash pine growing areas (Appendix 1); incubation in a chamber for 24 hours (20.5°C and 97% humidity); incubation in greenhouse (20°C and 12hr photoperiod); and evaluation of symptom expression (six months after inoculation).

The six seedlots were inoculated in each of four consecutive weeks during each of three different seasons of the year (i.e., winter, summer and fall inoculation) (Appendix 2). The winter inoculation (season 1) was characterized by the lowest ambient temperatures and shortest days during sowing, transplanting, and inoculation, with seedlings growing under continuously increasing ambient temperatures and day length until the beginning of the summer. Artificial light and heat were required for the first 6 months. The summer inoculation (season 2) was

characterized by low ambient temperatures during sowing and transplanting operations, and higher temperatures and the longest days during the inoculation, with the seedlings growing under continuously decreasing ambient temperatures and day length until the beginning of winter. Artificial light and heat were required for the last 3 months before evaluation. The fall inoculation (season 3) was characterized by higher ambient temperatures during sowing, transplanting and inoculation with the seedlings growing after inoculation under low ambient temperatures and short days. Artificial light and heat were required for all 6 months after inoculation.

For each week within a season, 120 seedlings of each of the 6 seedlots were inoculated. The 120 seedlings were divided into six groups of 20 seedlings each (a plot), and were inoculated in two runs (3 groups each) one day apart. The inoculated seedlings were placed in a humid chamber for 24 hr and were then randomly placed in the greenhouse in six blocks (one group per block). Thus, a total of 720 (20 seedlings x 6 blocks x 6 seedlots) were inoculated in each week of each season (8,640 total seedlings).

Six months after inoculation the presence (1) or absence (0) of several symptoms traits were evaluated (Table 1.2). Also, the number of adventitious shoots present in the galled area and the number of galls per seedling were recorded. Traits specifying gall characteristics were evaluated on the most prominent gall on each seedling (the normal RSC procedure) and on all galls present on each seedling. The multiple gall evaluation was conducted to ascertain if the evaluation of a single gall per seedling is sufficient to express the degree of resistance of a given family. Five traits (FAR, FAT, ENR, RES and RSS) were constructed combining information from more than one individual trait.

Analysis of variance (Table 1.3) was conducted on plot means for each symptom trait. For traits HEA, SYM, GAL, PGA, ENR, and RSS, a plot mean

Table 1.2. Name, abbreviation and description of the different traits evaluated six months after inoculation of slash pine seedlots with fusiform rust fungus.

Name	Abbreviation	Description	Reference
Healthy	HEA[a]	Seedling showing no rust symptoms.	Walkinshaw et al. (1980)
Symno	SYM[a]	Seedling showing area of purplish discoloration and/or needle base with purple discoloration.	Walkinshaw et al. (1980)
Gall	PGA[a]	Seedling with swellings or bumps on the stem.	Walkinshaw et al. (1980)
GAL		Number of galls present on seedling.	
Fat Gall	FR2[a] , FT2[c]	Gall that is twice the diameter of the stem if it were not galled.	Walkinshaw and Anderson (1983)
Medium Gall	MED[a]	Gall that is more than 1/3 but less than twice the diameter of the stem if it were not galled.	-
Thin Gall	THN[a]	Gall that is 1/3 or less than the diameter of the stem if it were not galled.	-
Gall Size	FAR[b], FAT[c]	Gall classified as: 1 fat, 0.5 medium, and 0 thin.	-
Rough Gall	ROR[a], ROU[c]	Gall with an are of discoloration covering more than 50% of the gall surface making it rough.	Walkinshaw and Anderson (1983)
Short Gall	SHR[a], SHT[c]	Gall less than 25mm in length.	Hubbard (1981)
Typical Gall	TPR[a], TYP[c]	Gall with swelling around the entire circumference of the stem.	Walkinshaw and Anderson (1983)
Adventitious Shoots	ADR[b], ADV[c]	Number of adventitious shoots emerging at acute angles along the apical portion of the stem.	Hubbard (1981) and Layton (1985)
	PAD[a]	Galled seedling with adventitious shoots.	-
Entry	ENR	Seedling classified as: 2.5 healthy, 2 symno, 1 one gall, 0.5 two 2 galls, and 0 more than 2 galls.	-
Post-Entry	RES	Seedlings whose values is the sum of: 0.5 if no adventitious shoots, 1 if gall is short, 1 if gall is not fat, and 1 if gall is not typical, and zero otherwise. Range of values is 0 to 3.5 per seedling.	-
RSS	RSS	Seedling classified as: 0 non-rough, and long gall; 0.25 non-rough, and short gall; 0.5 rough, and long gall; 1 rough, and short gall; 2 symno only, and 3 healthy seedling.	-

[a] Binomial traits evaluated on single gall per seedling as presence (1) or absence (0) of the symptom.
[b] Traits evaluated only a single gall on each seedling.
[c] Traits evaluated on all galls on each seedling.

contained data from the 20 seedlings of the same seedlot. For the other traits a plot mean contained data from only the galled seedlings in the plot. For traits based on evaluation of all galls on a seedling (FT2, FAT, ROU, SHT, TYP, and ADV), plot means were obtained by first averaging by seedling and then by plot. The binomial traits SYM, HEA, PGA, FR2, MED, THN, ROR, SHR, TPR, and PAD were analyzed as the untransformed proportion of seedlings displaying the symptom (i.e., untransformed plot means), and as the logistic (Cox, 1970) and arcsin (Snedecor and Cochran, 1967) transformations of the plot means. F-tests were calculated for all effects (Table 1.3). Satterthwaite's approximate F-test (Milliken and Johnson, 1984) was used for effects with no exact test (i.e., season and week nested in season effects).

Whether differences in symptom expression among seedlots are due to the level of field resistance (i.e., susceptible, average, and resistant) or are mainly due to sampling within a given seedlot was determined by partitioning the sum of squares representing differences among the six seedlots into the portion due to differences among the three levels of field resistance with 2 degrees of freedom, and the portion due to replications within level of resistance with 3 degrees of freedom (i.e., two seedlots at each of the three levels). The mean square for the interaction between seedlots and season of inoculation with 10 degrees of freedom was used as denominator for the F-test for both partitions. To investigate the pattern of variation for each trait across the three levels of field resistance, the sum of squares due to level of resistance (with 2 degrees of freedom) was partitioned into two 1 degree of freedom orthogonal contrasts: linear and quadratic. Since the levels of resistance were not equally spaced (see mean breeding values in Table 1.1), the coefficients for the contrasts for susceptible, average and resistant seedlots were respectively, 132, 24 and -156 for the linear contrast and -0.625, 1 and -0.375 for the quadratic contrast (B. F. Swindel, personal communication).

Table 1.3. Form of analysis of variance, expected mean squares and partition of seedlot and seedlot x season sums of squares as was performed on the 432 plot means (6 blocks x 6 seedlots x 4 weeks x 3 seasons) for each trait.

Source of Variation	D.F.	Expected Mean Squares
Season	2	$\sigma_e^2 + 6\sigma_{cw}^2 + 24\sigma_{sc}^2 + 6\sigma_b^2 + 36\sigma_w^2 + 144\sigma_s^2$
Week(Season)	9	$\sigma_e^2 + 6\sigma_{cw}^2 + 6\sigma_b^2 + 36\sigma_w^2$
Block(Week, Season)	60	$\sigma_e^2 + 6\sigma_b^2$
Seedlots:	5	$\sigma_e^2 + 6\sigma_{cw}^2 + 24\sigma_{sc}^2 + Q(\text{Seedlots})$
Level of Resistance	2	
Linear Contrast	1	
Quadratic Contrast	1	
Replication(Level)	3	
Seedlot x Season:	10	$\sigma_e^2 + 6\sigma_{cw}^2 + 24\sigma_{sc}^2$
Level x Season	4	
Rep(Level) x Season	6	
Seedlot x Week(Season)	45	$\sigma_e^2 + 6\sigma_{cw}^2$
Seedlot x Blk(Week, Sea.)	300	σ_e^2

The sum of squares due to the interaction between seedlots and season was partitioned into the portion due to the interaction between level of resistance and season with 4 degrees of freedom, and the portion due to the interaction between replications nested in level of resistance and season with 6 degrees of freedom. The mean square due to the interaction between seedlots and weeks nested in season with 45 degrees of freedom was used for the denominator for the F-test for both effects.

Results and Discussion

Overall Means and Transformations

The overall test mean for incidence of galls (PGA) (averaged over all seedlots, seasons, weeks and blocks) was high (0.83), indicating successful inoculations (Table 1.4). The resistant seedlots had higher mean values for HEA, SYM, THN, ROU, ROR, SHT, SHR, ENR, RES and RSC and lower mean values for the other traits. These results agree with the suggestion of Walkinshaw and Anderson (1983) that SYM, SHT, SHR, ROU and ROR are indicative of resistance, while TYP and TPR are indicative of susceptibility, and the results of Layton (1985) indicating that adventitious shoots (ADV, ADR and PAD in this experiment) represent susceptible reactions.

When using untransformed plot means of the 22 traits evaluated statistically, significant interactions ($p < 0.05$) occurred for level of resistance x season (9 traits), replication within level x season (2 traits), and for seedlots x week within seasons of inoculation (12 traits). These significant interactions could result from change in rank of the seedlots across seasons or weeks, a response which greatly reduces the usefulness of the trait to predict field performance. Or the interactions could be due to change in scale effects (i.e., relative distance between seedlots) with no change in ranking. Interactions resulting from scale effects usually can be eliminated by using an appropriate transformation of the data (Finney, 1971).

The benefits of the logistic and arcsin transformations in inducing additivity of main effects (i.e., eliminating significant interactions) for the 10 binomial traits (see binomial traits Table 1.2) were evaluated. Eight out of the 10 binomial traits benefited from the logistic transformation either by eliminating significant interaction (at $p > 0.05$) for level x season (HEA, SYM, PGA, PAD, SHR, and TPR) or for seedlot x week within season (SYM, ROR, THN, and TPR). The only two binomial

Table 1.4. Overall test means and means for susceptible, average and field resistant slash pine seedlots for the 22 traits evaluated six months after artificial inoculation with fusiform rust fungus.

Trait[a]	Test Mean[b]	Seedlot Level of Field Resistance		
		Susceptible[c]	Average[c]	Resistant[c]
Healthy (HEA)	0.065	0.041	0.040	0.114
Symno (SYM)	0.105	0.051	0.058	0.205
No. Galls (GAL)	1.119	1.223	1.275	0.859
Prop. of galls (PGA)	0.830	0.908	0.901	0.682
All galls evaluated:				
No. Adv.shoots (ADV)	1.646	2.062	1.567	1.309
Gall Size (FAT)	0.557	0.628	0.601	0.443
Fat galls (FT2)	0.292	0.348	0.335	0.193
Rough galls (ROU)	0.545	0.473	0.556	0.606
Short gall (SHT)	0.194	0.119	0.172	0.291
Typical gall (TYP)	0.710	0.814	0.737	0.579
Single gall evaluated:				
No. Adv.shoots (ADR)	1.759	2.200	1.701	1.374
Adv. shoots (PAD)	0.666	0.757	0.681	0.540
Gall Size (FAR)	0.590	0.660	0.643	0.467
Fat galls (FR2)	0.323	0.383	0.377	0.209
Thin galls (THN)	0.144	0.064	0.092	0.275
Medium galls (MED)	0.533	0.554	0.530	0.516
Rough galls (ROR)	0.573	0.502	0.593	0.624
Short gall (SHR)	0.123	0.051	0.085	0.235
Typical gall (TPR)	0.786	0.895	0.831	0.632
Entry (ENR)	1.063	0.960	0.940	1.287
Post-Entry (RES)	1.184	0.894	1.036	1.623
RSS	0.677	0.471	0.533	1.028

[a] For trait definitions see Table 1.2.
[b] Averaged across seedlots, seasons, weeks, and blocks.
[c] Averaged across replications within level of resistance, seasons, weeks and blocks. Differences among levels of resistance were significant p=0.05 for all traits except MED.

traits (FR2 and MED) that did not benefit in some manner from transformation had no significant interaction either for untransformed or transformed data. Therefore, we recommend that plot means for all binomial traits be transformed to a logistic scale. The logistic transformation was chosen instead of arcsin because it reduced or eliminated interactions for larger number of traits. Henceforth, all statistical tests for binomial traits are on logistic scale even though means are presented as untransformed data.

Importance of Two-way Interactions

Among the 22 traits (i.e., 10 binomial on logistic scale and 12 untransformed plot means), the level of resistance x season interaction was statistically significant ($p < 0.05$) only for GAL, FT2, and ENR. To examine the importance of these interactions, graphs were plotted for all three traits. The plots showed that in no case did the level x season interaction result from rank change. That is, for all traits, the screening procedure ranked seedlots with different levels of resistance consistently season after season.

Replication within level x season interaction was significant ($p < 0.05$) only for two traits (HEA and ENR) out of the 22 traits. These significant interactions may be attributed to scale effects, and also to minor rank changes among the two replications within the same level of resistance (example for ENR Figure 1.1). In addition, these two traits did not effectively discriminate susceptible from average seedlots, and some rank change occurred among the four means (two susceptible and two average seedlots) from season to season.

To better understand the seedlot x week within a season interaction, analyses of variance for all traits were conducted for each season of inoculation. The sum of squares due to seedlots x week was partitioned into the portion due to level of resistance x week with 6 degrees of freedom and the portion due to replication
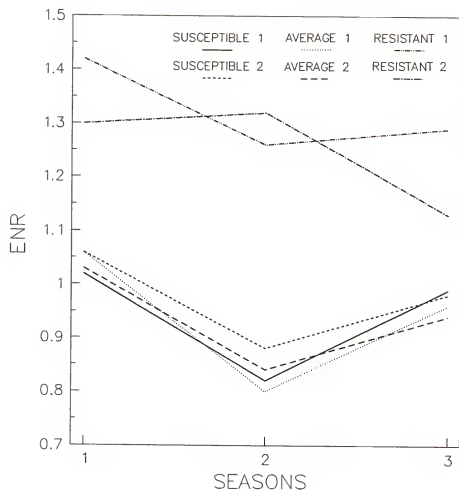
Figure 1.1. Effects of season of inoculation on expression of ENR on susceptible, average and field rust resistance slash pine seedlots after artificial inoculation with fusiform rust fungus. Seasons refer to (1) winter, 2) summer, and (3) fall inoculations. Replicate seedlots within each resistance level are labeled 1 and 2.

within level x week with 9 degrees of freedom. The level of resistance x week interaction was statistically significant ($p < 0.05$) only in two instances (ADV and SHT, first season) out of the 66 possibilities (22 traits tested in each of 3 seasons). These significant interactions, like those previously discussed, can be attributed to scale effect. The interaction between replication within level x week of inoculation was statistically significant ($p < 0.05$) for 1, 2 and 4 traits for the first, second and third season, respectively out of 22 possibilities in each season. These interactions occur randomly and never for the same trait in more than one season. Thus, they are not likely of biological importance.

In general, all the interactions presented for this data set are of minimal biological importance. That is, they did not result from rank change (season to season or week to week within a season) among seedlots with different levels of resistance. However, scale effect interactions did exist. So, to rank seedlots screened in different seasons or even in different weeks of the same season, the seedlot means should be adjusted for the scale effect of the specific test in which they were evaluated by 1) expressing the seedlots means as the deviation from the overall test mean, and 2) perhaps accounting for different variances in different tests.

Importance of Main Effects

Significant differences in mean level of trait expression from one week of inoculation to another were observed for five traits for the first season, nine for the second, and six for the third season of inoculation (out of 22 traits). In general for a given trait, differences among weeks of inoculation occurred randomly. A trait with a significant difference among weeks of inoculation for one season was not necessarily significant in another season. For the season main effect, the mean levels differed from season to season ($p < 0.05$) for all traits except for HEA, PGA,

FAR and THN (Table 1.5). Even though an attempt was made to standardize greenhouse conditions, variation of the ambient conditions (i.e., temperature, day length, light intensity and many others) may have affected the quality of the growing conditions for both the fungus and the seedlings resulting in more or less infection consequently increasing or decreasing the expression of a given trait. For example, seedlings inoculated during the winter (season 3) grew partially with artificial light and heat and had more infection. Thus, higher mean levels were realized for all traits expressing susceptibility. In general, seedlings in this season had larger variation in height, and smaller diameter than in either season 1 or 2. Also, for this season it was more difficult to manage uniform irrigation for all seedlings and a few died due to excess of water. It was common in a single plot (tray) to find some seedlings drying and others with excess water. Future tests will necessarily experience such environmental variations and consequently trait expression will vary from test to test. Thus, to compare results of families screened in different weeks or seasons, it will be imperative to adjust the family means for a given trait for scale effect of week and season in which they were tested.

Mean levels for seedlots with different levels of field rust resistance (i.e., susceptible, average and resistant) differed for all traits ($p < 0.05$) except for MED (Table 1.5). This trait might include galls that had stopped growing as well as those that later would become fat galls. Thus, unless this trait can be better characterized, it adds no information to discriminate among families with different levels of rust resistance and holds no promise for future investigation.

Significant differences for both linear and quadratic contrasts were observed for SYM, GAL, PGA, ADV, FAT, FAR, FR2, THN, ROR, ENR, RES and RSS (Table 1.5). Even though the quadratic response was significant, the sum of squares for linear accounted for more than 90% of the level sum of squares for all traits except for GAL, FR2, ROR and ENR. For all traits except ROR, the quadratic

Table 1.5. Summary of analysis of variance for the 22 symptom traits measured six months after artificial inoculation with fusiform rust fungus of slash pine seedlots of three levels of field rust resistance.

| | Mean Squares | | | | | |
| | Level of Resistance | | | | | Level $*$ Season |
Traits[a]	Total	Linear	Quadratic	R^{2c}	Season	
Healty(HEA)[b]	30.974**	55.395**	6.553ns	0.89	7.005**	1.324ns
Symno (SYM)[b]	85.289**	156.069**	14.50**	0.91	6.116*	1.184ns
No. Galls (GAL)	7.392**	11.460**	3.323**	0.78	3.999*	0.547**
Prop. Galls (PGA)[b]	104.392**	190.802**	17.83**	0.91	7.119ns	0.438ns
All galls evaluated:						
Adv.shoots (ADV)	21.107**	37.969**	4.245*	0.90	86.499**	0.977ns
Gall Size (FAT)	1.432**	2.696**	0.169*	0.94	0.374*	0.023ns
Fat galls (FT2)	1.073**	1.951**	0.194ns	0.91	1.389**	0.068*
Rough galls (ROU)	0.652**	1.199**	0.106ns	0.92	6.536**	0.054ns
Short gall (SHT)	1.122**	2.231**	0.013ns	0.99	0.933**	0.025ns
Typical gall (TYP)	2.049**	4.088**	0.011ns	0.99	0.707*	0.047ns
Single gall evaluated:						
Adv.shoots (ADR)	24.955**	46.507**	3.402ns	0.93	87.959**	0.966ns
Adv. shoots (PAD)[b]	43.348**	86.682**	0.013ns	0.99	56.640**	1.052ns
Gall Size (FAR)	1.638**	2.989**	0.287ns	0.91	0.223ns	0.022ns
Fat galls (FR2)[b]	31.725**	54.961**	8.488*	0.87	40.660**	1.209ns
Thin galls (THN)[b]	109.487**	206.664**	12.309**	0.94	6.317ns	0.728ns
Medium galls (MED)[b]	1.589ns	2.901ns	0.277ns	0.91	32.098**	1.757ns
Rough galls (ROR)[b]	13.614*	21.359**	5.868*	0.78	149.239**	1.697ns
Short gall (SHR)[b]	93.023**	181.315**	4.730ns	0.97	21.595**	1.930ns
Typical gall (TPR)[b]	95.870**	190.475**	1.264ns	0.99	28.271*	0.524ns
Entry (ENR)	5.491**	9.067**	1.915**	0.83	0.932*	0.184**
Post-Entry (RES)	21.528**	41.423**	1.633*	0.96	6.780**	0.232ns
RSS	13.428**	24.847**	2.009**	0.93	3.798**	0.082ns

[a] For trait definitions see Table 1.2.
[b] Binomial traits evaluated on logistic scale.
[c] R^2 express the portion of the total variability among the three levels of resistance accounted for by the linear relationship between the trait expression and field levels of resistance.
** Effect is significant at 0.01 level; * Effect is significant at 0.05 level; ns Effect is nonsignificant.

effect arose because the results for the average seedlot were closer to those for the susceptible ones than predicted by the linear regression on field breeding values (Table 1.4). For these traits, the greenhouse test discriminated well between resistant and other seedlots but not between average and susceptible (see Figure 1.2 with PGA as an example). For ROR, the average seedlots were closer to the resistant ones than predicted by linear regression on breeding values. Traits like ROR might be useful for separating highly susceptible families from the rest. As breeding programs advance, seedlots screened will be more resistant. Thus, traits like ROR might not help to separate families, while GAL, FR2, and ENR may be quite useful.

Linear response, but not quadratic, was significant ($p < 0.05$) for HEA, FT2, ROU, SHT, TYP, ADR, PAD, SHR, and TPR (Table 1.5). We expected that traits with such a response would adequately discriminate across a broad range of field resistance (i.e., among susceptible, average, and resistant seedlots). Indeed, this was the case for SHT, TYP, ADR, PAD, SHR, and TPR (see Figure 1.2 with TPR as an example). However, HEA, FT2 and ROU did not fit this profile since they either did not discriminate among susceptible and average seedlots (HEA and FT2) or among average and resistant seedlots (ROU). The lack of significant quadratic contrast for HEA, FT2 and ROU was probably due to of the lack of power of the F-test resulting from the use of a larger seedlot x season interaction as the error term for the F-test, when indeed we had assumed that this interaction was very small.

Promising Traits for Predicting Field Rust Resistance

A trait was considered promising for predicting field performance when it satisfied all or some of the following conditions: 1) it consistently ranked seedlots with different levels of field rust resistance from week to week and from season to
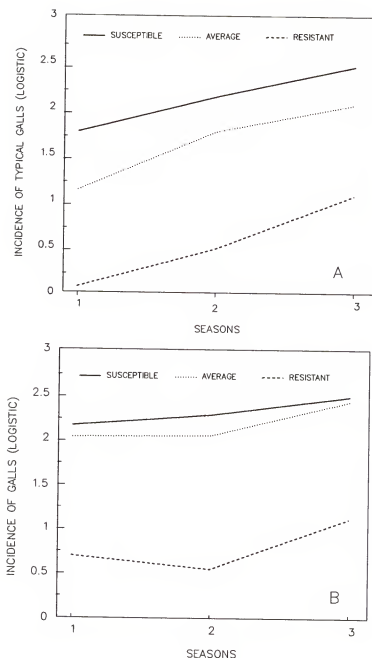
Figure 1.2. Effects of season of inoculation on (A) incidence of typical galls (TPR) and (B) gall incidence (PGA) on susceptible, average and field rust resistance slash pine seedlots after artificial inoculation with fusiform rust fungus. Seasons refer to (1) winter, (2) summer, and (3) fall inoculations. Note that for gall incidence, a significant quadratic effect arose because susceptible and average seedlots ranked more closer than predicted based on their field breeding values.

season of inoculation (i.e., the interactions between level of resistance and week or level of resistance and season of inoculation were either non-significant or could be attributed to scale effect); 2) it discriminated among seedlots with different field levels of rust resistance, 3) it was linearly related to field breeding values, and 4) it required less work to evaluate.

All 22 traits evaluated, except MED, consistently ranked susceptible, average and field resistant seedlots from week to week and season to season, thus satisfying condition 1. The second and third conditions depend on the overall goal of the test. If the goal was to discriminate susceptible, average and resistant seedlots, SHT, SHR, TYP, TPR, ADR and PAD satisfied both the second and third conditions. That is, these traits are linearly related to seedlot field level of resistance and efficiently discriminated among susceptible, average and resistant seedlots. The fourth condition involves economic reasons. Based on current knowledge, evaluation of a single gall per seedling yielded as reliable information as evaluation of all galls on a given seedling. However, these results might change as knowledge of the mechanisms involved in fusiform rust resistance improves.

In summary, the traits SHR, TPR, PAD and ADR, which ranked the seedlots uniformly across weeks and seasons of inoculation, discriminated among seedlots with different level of rust resistance, were linearly related to field breeding values, and were evaluated only in one gall per seedling, are recommended as the most promising traits to be included in selection index. In addition, the traits HEA, SYM, PGA, THN, FAR, FR2, GAL, ENR, RES and RSS should be considered especially if the goal is mainly to discriminate among average and resistant seedlots.

Thus, it appears that several traits are available that when combined in a selection index might allow precise and consistent prediction of field breeding values from RSC results.

Conclusions

The results indicated that most traits consistently ranked seedlots according to their field resistance across seasons and weeks of inoculation and there are many promising traits that when combined in selection index might allow precise and consistent prediction of field breeding values from greenhouse results (RSC). However, to compare seedlots tested in different seasons or even weeks (i.e., different tests), the seedlot means should first be adjusted for the scale effect of the test in which they were tested. For traits with a binomial distribution, transformation of the data to the logistic scale reduced interactions and should be considered as part of the screening procedure.

CHAPTER 2
ESTIMATION OF GENETIC PARAMETERS FOR GREENHOUSE TRAITS
ON SLASH PINE FAMILIES ARTIFICIALLY INOCULATED WITH
FUSIFORM RUST FUNGUS

Introduction

A topic of great importance among tree breeders is how to reduce the number of years required for selection, testing and breeding of southern pines. For fusiform rust resistance, the time required for testing could be greatly reduced if the usual field progeny tests were replaced by artificial testing under greenhouse conditions. Such a technique, the concentrated basidiospore system, has been used since 1973 at the USDA Forest Service Resistance Screening Center (RSC), to operationally screen large numbers of slash and loblolly pine families for fusiform rust resistance. Studies conducted throughout the years have provided evidence that this early testing technique is feasible (Jewell, 1960; Dinus, 1969; Miller, 1970; Powers et al., 1971; Matthews et al., 1978; and Anderson et al., 1983) and have identified several traits that, when combined in a selection index, might allow precise and consistent prediction of field breeding values from RSC results (Walkinshaw et al., 1980; Hubbard, 1981). However, to best use this technique to predict field breeding values, the quantitative genetic parameters of the greenhouse traits should be known.

Using 17 full-sib slash pine families and results of a single greenhouse test, Layton (1985) showed that several fusiform rust symptoms were heritable traits. For example, three out of the 11 symptoms evaluated had individual tree

24

heritabilities greater than 0.30. In the first chapter of this dissertation, we showed the RSC technique ranked susceptible, average, and rust resistant slash pine seedlots consistently across tests conducted in different weeks and seasons; however, overall test means varied from test to test for several traits, and it is possible that estimated genetic parameters (i.e., heritability, family variance, variance of family means) also vary among tests. In addition, estimated genetic parameters and the magnitudes of genotype x environment interaction might differ among sets of families (i.e., families from different locations).

The overall goal of this chapter was to identify promising greenhouse traits to predict field performance, i.e., traits with low family x test interaction, high heritabilities, stable variances of family means across tests and for different sets of families, and traits not strongly correlated with other promising traits. The specific objectives were: 1) To determine the importance of family x test interaction for 16 greenhouse traits, 2) To estimate genetic parameters (i.e., family variance, variance of family means, and family heritability) for each trait, 3) To estimate genetic correlations among the traits, and 4) To determine if the estimated genetic parameters vary among different greenhouse tests and among four independent samples of families.

Materials and Methods

Sampling

A total of one hundred open-pollinated families were obtained from two populations of slash pine parents in the Florida Cooperative Forest Genetics Research Program (CFGRP). Population 1 consists of 1397 parents selected for growth, but not for resistance to fusiform rust. The breeding values for rust resistance for each parent in this population were predicted from data from one to

20 field progeny tests and are expressed as R50, which is the expected rust incidence that offspring of a given parent will have when average material experiences 50% infection. These R50's are approximately normally distributed with mean 50 and standard deviation 22 (White and Hodge, 1987). The parents chosen to represent this population were required to be tested in at least four field progeny tests. An inventory of the 1397 parents showed that 523 parents met this condition. The distribution of breeding values (R50) for this subset of the population has first and second moments similar to the overall population. From this subset of population 1, three groups of parents (i.e, groups 1, 2, and 3) were randomly chosen for this study (Table 2.1). The parents in each group were from a first-generation clonal orchard, and the mean breeding value of the sample for each group was kept similar to the average breeding value of all clones in the orchard. The three groups of parents can be classified according to the average parental breeding values as two groups of average rust resistance (groups 2 and 3), and one group slightly more resistant than average (group 1).

The fourth group of 25 parents was randomly selected from population 2, a seed production area in Wayne Co, Georgia, containing 278 trees. This area was a commercial plantation with high incidence of fusiform rust (90 to 95% of the trees infected) in which rust-infected trees were removed. Hodge et al. (1989) showed that mass selection of rust-free individuals in this area resulted in significant realized genetic gains. Also, the remaining rust-free trees were progeny tested, and the results indicated that they were more resistant on average than parents in unimproved population 1 (Goddard et al., 1975). The parental breeding values (predicted from open-pollinated field test data using best linear prediction) for this population are approximately normally distributed with a mean R50 of 36 and standard deviation 14. Thus, according to the average parental breeding values the fourth group (Table 2.1) was classified as a resistant group.

Table 2.1. Characteristics of the four groups of 25 slash pine parents and the seed orchards from where the seeds were collected.

| Groups | Pop.[a] | Mean R50[b] | Seed Orchard | | |
			Location	Date of Establishment	Mean R50[b,c]
1	1	39.5 ± 20.0	Perry,FL	1957-62	42.0
2	1	49.1 ± 19.8	Yulee,FL	1957-70	48.0
3	1	51.9 ± 18.5	Munson,FL	1963	40.0
4	2	31.7 ± 17.3	Wayne,GA	1965	36.0

[a] Population 1 is represented by 1397 parents unselected for rust with mean R50 of 50 and standard deviation of 22. Population 2 is a seed production area in Wayne, GA, represented by 278 parents selected for freedom from rust in a highly infected stand with mean R50 of 36 and standard deviation of 14.

[b] R50, the predicted parental breeding values were obtained by best linear prediction using results from several open-pollinated field progeny tests in the Cooperative Forest Genetics Research Program. Each breeding value predicts the average percentage of rust infection in a field environment where unimproved checks (i.e., average material) would incur 50% infection (White and Hodge, 1987).

[c] Orchard mean R50 was weighted by the number of ramets of each clone, except for the seed production area at Wayne, GA.

Greenhouse Tests

The experiment was conducted at the RSC using their standard concentrated basidiospore suspension technique (Anderson et al., 1983; Knighten et al., 1988). In brief, the procedures were: seed stratification; seed sowing in 10 x 15 x 5 cm germination trays in the greenhouse; transplanting germinants into Ray Leach tubes (three weeks after sowing); seedling inoculation (five weeks after transplanting) with a basidiospore suspension (20,000 spores per ml) composited from spores collected

in five slash pine growing areas (Appendix 1); incubation in a chamber for 24 hours (20.5°C and 97% humidity); and incubation in the greenhouse (20°C and 12hr photoperiod) until evaluation six months after inoculation.

Each group of 25 families was tested three times during the year (i.e., winter, summer and fall). To maintain standard greenhouse conditions (20°C and 12 hr photoperiod), artificial light, heat and cooling were supplied as required. The description of the environmental conditions during each testing period are presented in chapter 1. In each test, 120 seedlings of each family were inoculated. The 120 seedlings were divided into six sets of 20 seedlings each (a plot), and were inoculated in two runs (3 sets each) one day apart. The inoculated seedlings were placed in a humid chamber for 24hr and then were randomly placed in the greenhouse in six blocks (1 set per block and 3 blocks per run). Thus, a total of 3,000 seedlings (20 seedlings x 25 families x 2 runs x 3 blocks) were inoculated in each test for each group, a total of 36,000 seedlings in the entire experiment (3,000 seedlings x 4 groups x 3 testing periods).

Six months after inoculation the presence (1) or absence (0) of several fusiform rust symptoms (i.e., greenhouse traits) were evaluated (Table 2.2). Also, the number of adventitious shoots present in the galled area, the number of galls per seedling and the traits FAR, ENR, RES and RSS (constructed by combining several individual traits) were evaluated. Traits specifying gall characteristics were evaluated on the most prominent gall on each seedling (the normal RSC procedure) and on all galls on each seedling. The traits based on multiple gall evaluation are not discussed here since initial analyses showed that evaluation of a single gall per seedling yielded as reliable information as evaluation of all galls on a seedling.

Table 2.2. Name, abbreviation and description of the 16 traits evaluated six months after inoculation of slash pine seedlots with fusiform rust fungus.

Name	Abbreviation	Description
Initial Infection:		
Healthy	HEA[a]	Seedling showing no rust symptoms.
Symno	SYM[a]	Seedling showing area of purplish discoloration and/or needle base with purple discoloration.
Gall	PGA[a] GAL	Seedling with swellings or bumps on the stem. Number of galls present on seedling.
Entry	ENR	Seedling classified as: 2.5 healthy, 2 symno, 1 one gall, 0.5 two galls, and 0 more than 2 galls.
Colonization:		
Adventitious Shoots	ADR	Number of adventitious shoots emerging at acute angles along the galled portion of the stem.
	PAD[a]	Galled seedling with presence of adventitious shoots.
Fat Gall	FR2[a]	Gall that is twice the diameter of the stem if it were not galled.
Gall Size	FAR	Gall classified as: 1 fat, 0.5 medium, and 0 thin.
Thin Gall	THN[a]	Gall that is 1 1/3 or less than the diameter of the stem if it were not galled.
Medium	MED[a]	Gall that is more than 1 1/3 but less than twice the diameter of the stem if it were not galled
Rough Gall	ROR[a]	Gall with an area of discoloration covering more than 50% of the gall surface making it rough.
Short Gall	SHR[a]	Gall less than 25mm in length.
Typical Gall	TPR[a]	Gall with swelling around the entire circumference of the stem.
Post-Entry	RES	Seedlings whose values is the sum of: 0.5 if no adventitious shoots, 1 if gall is short, 1 if gall is not fat, and 1 if gall is not typical, and zero otherwise. Range of values is 0 to 3.5 per seedling.
Complex:		
RSS	RSS	Seedling classified as: 0 non-rough long gall, 0.25 non-rough short gall, 0.5 rough long gall, 1 rough short gall, 2 symno only, and 3 healthy seedling.

[a] Binomial traits evaluated as presence (1) or absence (0) of the symptom. All binomial traits were transformed to the logistic scale for all analyses.

<u>Analyses</u>

Analysis of variance (Table 2.3) was conducted on plot means for each greenhouse trait for each group. The binomial traits (SYM, HEA, PGA, PAD, FR2, MED, THN, ROR, SHR, and TPR) were analyzed after logistic transformation of the plot means as suggested in chapter 1. F-tests were calculated for family and family x test effects, and the Satterthwaite's approximate F-test (Milliken and Johnson, 1984) was used for effects which had no exact test (i.e., test effect).

<u>Estimation of Genetic Parameters</u>

Variance components for the combined analyses across testing periods were estimated by equating observed mean squares to their expected values and solving the system of equations. Variance of family means ($Var(\overline{y})$) and family heritability (h_f^2) for each greenhouse trait were estimated for each group combined across the three tests as follows:

$$Var\ (\overline{y}) = \sigma_f^2 + \sigma_{ft}^2/3 + \sigma_{fn}^2/6 + \sigma^2/18, \text{ and}$$

$$h_f^2 = \sigma_f^2 \ / \ (\sigma_f^2 + \sigma_{ft}^2/3 + \sigma_{fn}^2/6 + \sigma^2/18)$$

where 3 is the number of tests, 6 is the number of runs x tests (i.e., 2 runs x 3 tests), 18 is the number of runs x blocks x tests (i.e., 2 runs x 3 blocks x 3 tests). Standard deviations for family variance (σ_f^2), variance of family means ($Var\ (\overline{y})$), and family heritability (h_f^2) were estimated as given by Namkoong (1979 pp.232-3).

Genetic correlations between same trait measured in two different environments are a useful measure of importance of genotype x environment interactions (in this study family x test interaction) (Dickerson, 1962; Yamada, 1962; Falconer, 1981; Eisen and Saxton, 1983; Eisen, 1987; and Bulmer, 1985). In this experiment genetic correlation between the same trait measured in two different tests was estimated using the framework of analysis of variance for each group and

Table 2.3. Analysis of variance and expected mean squares as performed on the 450 plot means (2 runs x 3 blocks x 25 families x 3 tests) for each trait and for each group of slash pine families.

Source of Variation	D.F.	Expected Mean Squares
Test	2	$\sigma^2 + 3\sigma_{frt}^2 + 6\sigma_{ft}^2 + 25\sigma_{rbt}^2 + 75\sigma_{rt}^2 + 150\sigma_t^2$
Run(Test)	3	$\sigma^2 + 3\sigma_{frt}^2 + 6\sigma_{ft}^2 + 25\sigma_{rbt}^2 + 75\sigma_{rt}^2$
Run x Block(Test)	12	$\sigma^2 + 25\sigma_{rbt}^2$
Family	24	$\sigma^2 + 3\sigma_{frt}^2 + 6\sigma_{ft}^2 + 18\sigma_f^2$
Family x Test	48	$\sigma^2 + 3\sigma_{frt}^2 + 6\sigma_{ft}^2$
Family x Run(Test)	72	$\sigma^2 + 3\sigma_{frt}^2$
Error	288	σ^2

trait combined across tests as follows:

$$r_g = \sigma_f^2 / (\sigma_f^2 + \sigma_{ft}^2)$$

where

r_g = estimated genetic correlation,

σ_f^2 = estimated family variance from ANOVA for a given trait , and

σ_{ft}^2 = estimated family x test variance from ANOVA for a given trait.

A genetic correlation of one or nearly one indicates that the interaction is negligible. On the other hand, a genetic correlation significantly less than one could be either due to change in rank of families in different tests, or due to differences between tests in scale of the genetic effects (i.e., heterogenous family variance from test to test for a given trait). As suggested by Dickerson (1962) and Yamada (1962), a correction for the heterogeneity of variances among tests can be made before computing the genetic correlation. For this study the correction term is

$K = [(\sigma_{F1} - \sigma_{F2})^2 + (\sigma_{F1} - \sigma_{F3})^2 + (\sigma_{F2} - \sigma_{F3})^2] / 6$, where σ_{F1}, σ_{F2}, and σ_{F3} are estimated family standard deviations for each trait and group from analyses of variance conducted separately for testing periods 1, 2, and 3, respectively. The correction term, 'K' is then subtracted from σ_{fs}^2 resulting in σ_{fs*}^2, and the new genetic correlation estimated as $r_g^* = \sigma_f^2 / (\sigma_f^2 + \sigma_{fs*}^2)$. This adjusted genetic correlation is free of bias due to interaction resulting from scale effects, and for balanced data sets with no negative estimates of variance components is equal to the average type B genetic correlation as estimated by Burdon (1977). Standard deviation estimates for the unadjusted genetic correlation (r_g) were estimated as given by Mode and Robinson (1959) and used as approximate standard deviations for the adjusted genetic correlation (r_g^*). An approximate 95% confidence interval was estimated for all r_g^* (i.e., $r_g^* \pm 2$ x standard deviation of the unadjusted correlation).

Genetic correlations were also estimated between all pairs of traits measured on the same individuals using the framework of the analysis of covariance combined across all tests (analogous to the ANOVA in Table 2.3) as follows:

$r_{g(i,j)} = \sigma_{f(i,j)} / (\sigma_{fi}^2 \text{ x } \sigma_{fj}^2)^{0.5}$

where

$r_{g(i,j)}$ = estimated genetic correlation for traits i and j,

$\sigma_{f(i,j)}$ = estimated family covariance between traits i and j from the analysis of covariance combined across tests,

σ_{fi}^2 = estimated family variance of trait i, from ANOVA (Table 2.3),

σ_{fj}^2 = estimated family variance of trait j, from ANOVA (Table 2.3).

<u>Results and Discussion</u>

<u>Test Means</u>

The overall test means (untransformed scale) for each group of 25 slash pine families (averaged over families, tests, blocks and runs) for incidence of galls (PGA) were 0.82, 0.93, 0.83 and 0.73 for groups 1, 2, 3, and 4, respectively, indicating successful inoculations. Among the 16 traits evaluated (Table 2.2), five traits were related to initial infection, 10 traits characterized initial infection and subsequent response of the tree to colonization (severity of infection), and one trait was a mixture of initial infection plus subsequent colonization (complex trait). Since, selection under field conditions was based on disease incidence, we expected larger differences among the groups for traits related to initial infection rather than for those traits related to severity of infection. Indeed, differences in test means occurred for HEA, SYM, GAL, PGA, and ENR (traits related to initial infection), and RSS (complex trait) between group 2 (average group from unimproved population) compared to group 4 (resistant group from selected population), with groups 1 and 3 intermediate. For many traits differences among groups of families seems unrelated to previous selection for rust resistance (Table 2.4).

<u>Family by Test Interactions</u>

Of the 16 traits evaluated for each group, statistically significant family x test interactions ($p < 0.05$) occurred for 8, 7, 2, and 12 traits for groups 1, 2, 3, and 4, respectively. However, statistical significance alone is not enough to determine the practical importance of interactions (Squillace, 1969; Matheson and Raymond, 1986). Significant interaction could result from change in rank of the families in different greenhouse tests, a response that greatly reduces the usefulness of the trait to predict field performance. Or the interaction could be due to change in scale

Table 2.4. Test means (averaged across 2 runs, 3 blocks, 3 seasons and 25 families) for the 16 traits evaluated for four groups of 25 slash pine families six months after artificial inoculation with fusiform rust fungus.

Traits[a]	Groups			
	1	2	3	4
Healthy (HEA)	0.07	0.03	0.07	0.10
Symno (SYM)	0.11	0.04	0.10	0.17
No. galls (GAL)	1.17	1.15	1.14	0.95
Prop. of galls (PGA)	0.82	0.93	0.83	0.73
Prop. adv. shoots (PAD)	0.66	0.74	0.67	0.58
No. adv. shoots (ADR)	1.77	2.39	1.65	1.74
Fat galls (FR2)	0.25	0.32	0.32	0.39
Gall size (FAR)	0.53	0.60	0.60	0.63
Thin galls (THN)	0.18	0.11	0.11	0.13
Medium galls (MED)	0.57	0.57	0.56	0.48
Rough galls (ROR)	0.56	0.60	0.51	0.56
Short galls (SHR)	0.15	0.09	0.08	0.14
Typical galls (TPR)	0.80	0.83	0.85	0.75
Entry (ENR)	1.05	0.98	1.06	1.21
Post-entry (RES)	1.27	1.07	1.06	1.21
RSS	0.71	0.48	0.65	0.88

[a] For trait definitions see Table 2.2.

(i.e., heterogenous variances among tests) with no change in rank of families; this has very little biological importance. However, heterogeneity of variances should be quantified if families screened in different tests are going to be used for predictive purposes.

To better understand the nature and importance of the family x test interactions, the concept of genetic correlation adjusted for heterogeneity of variances among tests was used (Dickerson, 1962; Yamada, 1962; Falconer, 1981; Eisen and Saxton, 1983; and Bulmer, 1985). In brief, the concept implies that if interaction results only from heterogeneity of variances among tests (i.e., scale effects), the adjusted genetic correlation is one or nearly one. Genetic correlations less than one indicate that interactions have resulted from change in family rank among tests. For the trait SYM, the adjusted genetic correlation (r_g^*) was greater than 0.5 and not different from one (p = 0.05) for all four groups of families (Table 2.5); further, the average over all four groups was 0.96, indicating that interaction for this trait was of no biological or practical importance. For the traits GAL, PGA, PAD, ADR, THN, SHR, TPR, ENR, RES, and RSS, the adjusted genetic correlations three out of the four groups of families (2 in the case of PAD) were significantly greater than 0.5 and not significantly different from one (p = 0.05) (Table 2.5). Also, for these traits, all correlations were greater than zero, and the average correlation (across the four groups) ranged from 0.83 to 0.92. Thus, even though family x test interaction for these traits was measurable and resulted family rank changes for at least one of the four groups, in general, these interactions were not important, and should not limit the usefulness of these traits to predict field performance.

In contrast, for the remaining five traits (HEA, FAR, FR2, ROR, and MED), out of the 20 possible trait-group combinations, the adjusted genetic correlation was not different from zero (p = 0.05) seven times. This suggests that

Table 2.5. Adjusted genetic correlations for the same trait measured in different tests (r_g^*) for each of the 16 traits evaluated for four groups of 25 slash pine families six months after artificial inoculation with fusiform rust fungus.

Traits[a]	Groups 1 r_g^* ± STD[c]	2 r_g^* ± STD[c]	3 r_g^* ±STD[c]	4 r_g^* ±STD[c]	Overall Mean ± SE[d]	Number of tests[e] 0.5≤r_g^*≤1	r_g^*=0
Health (HEA)[b]	0.87 ±0.10	0.22 ±0.24	0.78 ±0.17	0.94 ±0.09	0.70 ±0.16	2	1
Symno (SYM)[b]	1.00 ±0.08	0.90 ±0.12	1.00 ±0.08	0.95 ±0.05	0.96 ±0.02	4	0
No. galls (GAL)	1.00 ±0.07	0.58 ±0.16	1.00 ±0.07	0.92 ±0.04	0.88 ±0.10	3	0
Prop. of galls (PGA)[b]	0.97 ±0.06	0.69 ±0.16	1.00 ±0.05	0.95 ±0.04	0.90 ±0.07	3	0
Prop. adv. shoots (PAD)[b]	0.84 ±0.11	0.69 ±0.12	0.91 ±0.14	0.92 ±0.30	0.84 ±0.05	2	0
No. adv. shoots (ADR)[b]	0.87 ±0.10	1.00 ±0.08	0.86 ±0.09	0.63 ±0.15	0.84 ±0.08	3	0
Fat galls (FR2)[b]	0.03 ±0.30	1.00 ±0.64	0.94 ±0.25	0.85 ±0.11	0.71 ±0.23	1	2
Gall size (FAR)	0.47 ±0.26	1.00 ±0.20	0.73 ±0.21	0.89 ±0.08	0.77 ±0.12	2	1
Thin galls (THN)[b]	0.96 ±0.14	0.82 ±0.14	1.00 ±0.37	0.88 ±0.11	0.92 ±0.04	0	1
Medium galls (MED)[b]	0.80 ±0.22	1.00 ±7.34	0.94 ±0.30	0.65 ±0.23	0.85 ±0.08	0	1
Rough galls (ROR)[b]	1.00 ±0.75	0.53 ±0.20	0.38 ±0.23	0.85 ±0.13	0.69 ±0.14	1	2
Short galls (SHR)[b]	0.95 ±0.09	1.00 ±0.12	1.00 ±0.25	0.56 ±0.20	0.88 ±0.11	3	0
Typical galls (TPR)[b]	0.83 ±0.11	1.00 ±0.11	0.99 ±0.25	0.69 ±0.19	0.88 ±0.07	3	0
Entry (ENR)	1.00 ±0.05	0.54 ±0.15	1.00 ±0.04	0.93 ±0.03	0.87 ±0.11	3	0
Post-entry (RES)	0.79 ±0.12	1.00 ±0.10	0.74 ±0.21	0.80 ±0.13	0.83 ±0.06	3	0
RSS (RSS)[b]	1.00 ±0.04	0.70 ±0.14	1.00 ±0.03	0.93 ±0.03	0.91 ±0.07	3	0

[a] For trait definitions see Table 2.2; [b] Binomial traits evaluated on logistic scale.
[c] Standard deviation estimated for the unadjusted genetic correlation; [d] Standard error of the mean among the four estimates of r_g^*.
[e] Number of tests for which column 1: the genetic correlation is greater than 0.5 and not significantly different from 1 and column 2: r_g^* was not significantly different from zero (p = 0.05).

interaction for these traits did not result from scale effects, and that the families may indeed be changing ranks from test to test. Therefore, unless these interactions can be biologically and/or technically predicted, the usefulness of these traits to predict field performance is somewhat reduced. If these traits are used in a predictive index, it might be necessary to test a given set of families in multiple testing periods. For MED, it is possible that galls scored as medium include all galls that had stopped growing (true mediums) as well those that would continue to grow and would later became fat galls. Consequently the precision is low and family x test interaction is high. For the trait HEA, the lower correlation (i.e., r_g^* not different from zero) observed for the group 2, might be a consequence of the low incidence of healthy seedlings for the families in this group (i.e., 0.03 on the average, Table 2.4). On the other hand, Layton (1985) suggested that healthy seedlings (HEA) may be escapes. For the remaining traits (FR2, FAR, and ROR) it is possible that their expression is more related to the virulence of the fungus rather than to the genotype of the seedlings (C. Walkinshaw, personal communication).

Family Variance, Variance of Family Means and Heritability Estimates

From the ANOVAs combined across tests, four estimates of family variance (σ_f^2), variance of family means ($Var(\overline{y})$), and family heritability (h_f^2) were obtained for each trait (i.e., one estimate for each group of families). For all traits, both family variance (σ_f^2) and variance of family means ($Var(\overline{y})$) varied between at least two of the four groups of families. In general, for the traits related to initial infection (except HEA), and for the complex trait RSS, family variance (Table 2.6) and variance of family means (results not shown) were larger for the resistant group (group 4). These larger variances for group 4 may be associated with the fact that families in this group were more rust resistant with the overall test mean closer to

Table 2.6. Family variance estimates (σ_f^2) for the four groups of 25 slash pine families six months after artificial inoculation with fusiform rust fungus.

Traits	Groups				MEAN ± SE[c]
	1	2	3	4	
Healthy (HEA)[a]	0.232 ±0.078[b]	0.010 ±0.015[b]	0.119 ±0.048[b]	0.217 ±0.070[b]	0.144 ±0.051
Symno (SYM)[a]	0.361 ±0.112	0.140 ±0.048	0.312 ±0.099	0.860 ±0.255	0.418 ±0.155
No. galls (GAL)	0.031 ±0.010	0.004 ±0.002	0.024 ±0.008	0.064 ±0.019	0.031 ±0.012
Prop. of galls (PGA)[a]	0.413 ±0.130	0.112 ±0.045	0.364 ±0.111	0.929 ±0.272	0.455 ±0.171
Prop. adv. shoots (PAD)[a]	0.167 ±0.058	0.159 ±0.058	0.096 ±0.001	0.059 ±0.025	0.120 ±0.026
No. adv. shoots (ADR)[a]	0.148 ±0.050	0.251 ±0.078	0.149 ±0.050	0.063 ±0.036	0.153 ±0.038
Fat galls (FR2)[a]	0.001 ±0.030	0.128 ±0.048	0.139 ±0.057	0.228 ±0.076	0.124 ±0.047
Gall size (FAR)	0.001 ±0.001	0.003 ±0.001	0.002 ±0.001	0.006 ±0.002	0.003 ±0.001
Thin galls (THN)[a]	0.211 ±0.073	0.178 ±0.063	0.076 ±0.033	0.216 ±0.072	0.170 ±0.033
Medium gall (MED)[a]	0.060 ±0.028	0.037 ±0.017	0.077 ±0.033	0.055 ±0.028	0.057 ±0.008
Rough gall (ROR)[a]	0.117 ±0.042	0.078 ±0.038	0.034 ±0.027	0.177 ±0.061	0.102 ±0.030
Short gall (SHR)[a]	0.289 ±0.095	0.240 ±0.073	0.092 ±0.035	0.061 ±0.030	0.171 ±0.056
Typical galls (TPR)[a]	0.226 ±0.078	0.185 ±0.060	0.072 ±0.028	0.081 ±0.035	0.141 ±0.038
Entry (ENR)	0.026 ±0.008	0.002 ±0.001	0.021 ±0.006	0.058 ±0.017	0.027 ±0.012
Post-entry (RES)	0.040 ±0.014	0.040 ±0.012	0.009 ±0.004	0.039 ±0.014	0.032 ±0.008
RSS	0.061 ±0.019	0.006 ±0.002	0.047 ±0.014	0.119 ±0.034	0.058 ±0.023

[a] Binomial traits evaluated on logistic scale.
[b] Family variance (σ_f^2) ± standard deviation estimated from Namkoong (1979).
[c] Average across the four groups ± standard error of the mean among the four groups.

the middle range of the distribution than for the other three groups. This allows more spread of the data (especially for SYM and PGA and consequently for ENR and RSS) and facilitates expression of family differences. These results, to a certain extent, are similar to field data, where family variance (Sohn and Goddard, 1979) and variance of family means (White and Hodge, 1987) depend on the mean level of rust infection in the test, being highest between 40 and 70% infection.

For the traits expressing severity of infection (i.e., initial infection plus colonization), differences in variances among the four groups of families were smaller, and seemed not associated with the resistance level of the groups. This is consistent with the observation earlier (Table 2.4) that the overall trait means for the traits associated with severity of infection did not differ as much among the four groups as for the traits associated with incidence of infection.

Family heritability estimates of the greenhouse traits are important parameters in selecting traits to predict field performance (White and Hodge, in preparation). In general, the heritability estimate of a candidate trait should be high, reliable (i.e., with small standard deviation) and similar for different sets of families within the target population. In general, for this study, the estimated family heritabilities averaged across the four groups for all traits were relatively high (maximum = 0.90 and minimum = 0.58) and reliable (i.e., with small standard error of the mean among the four groups) (Table 2.7). The most heritable traits ($h_f^2 >$ 0.80) were SYM, GAL, PGA, ENR, RES, and RSS. Therefore, according to the suggestion of White and Hodge (1989) that higher heritability of indirect traits increases the precision of the prediction of the target trait, these traits are most promising for an index to predict field breeding values. On the other hand, the least heritable traits ($h_f^2 <$ 0.70) HEA, FR2, FAR, MED, and ROR, are less promising; but depending upon their correlations with field performance, they still might be valuable in a predictive index. However, in order to use these traits in

Table 2.7. Family heritability estimates (h_r^2) for the 16 traits evaluated for the four groups of 25 slash pine families six months after artificial inoculation with fusiform rust fungus.

Traits	Groups				MEAN ±SE[c]
	1	2	3	4	
Healthy (HEA)[a]	0.86 ±0.050[b]	0.23 ±0.264[b]	0.73 ±0.095[b]	0.86 ±0.046[b]	0.67 ± 0.150
Symno (SYM)[a]	0.93 ±0.025	0.81 ±0.064	0.91 ±0.031	0.94 ±0.022	0.90 ± 0.030
No. galls (GAL)	0.89 ±0.038	0.56 ±0.149	0.91 ±0.033	0.95 ±0.018	0.83 ±0.090
Prop. of galls (PGA)[a]	0.92 ±0.028	0.71 ±0.100	0.95 ±0.018	0.95 ±0.017	0.88 ± 0.058
Prop. adv. shoots (PAD)[a]	0.84 ±0.057	0.77 ±0.078	0.82 ±0.065	0.67 ±0.111	0.78 ± 0.038
No. adv. shoots (ADR)	0.86 ±0.051	0.89 ±0.038	0.86 ±0.049	0.52 ±0.165	0.78 ±0.088
Fat galls (FR2)[a]	0.02 ±0.350	0.75 ±0.086	0.72 ±0.098	0.83 ±0.056	0.58 ± 0.188
Gall size (FAR)	0.49 ±0.182	0.82 ±0.060	0.68 ±0.114	0.88 ±0.042	0.72 ±0.087
Thin galls (THN)[a]	0.84 ±0.057	0.79 ±0.073	0.69 ±0.111	0.84 ±0.054	0.79 ±0.035
Medium gall (MED)[a]	0.64 ±0.127	0.61 ±0.135	0.69 ±0.110	0.57 ±0.146	0.63 ±0.025
Rough gall (ROR)[a]	0.82 ±0.064	0.59 ±0.139	0.39 ±0.216	0.82 ±0.063	0.66 ± 0.104
Short gall (SHR)[a]	0.88 ±0.043	0.91 ±0.029	0.77 ±0.082	0.59 ±0.139	0.79 ± 0.072
Typical galls (TPR)[a]	0.84 ±0.058	0.87 ±0.045	0.75 ±0.048	0.66 ±0.177	0.78 ±0.047
Entry (ENR)	0.94 ±0.022	0.60 ±0.138	0.95 ±0.017	0.96 ±0.013	0.86 ±0.088
Post-entry (RES)	0.81 ±0.068	0.89 ±0.037	0.68 ±0.115	0.80 ±0.069	0.81 ±0.043
RSS	0.95 ±0.016	0.74 ±0.088	0.96 ±0.015	0.96 ±0.015	0.90 ±0.054

[a] Binomial traits evaluated on logistic scale.
[b] Family heritability (h_r^2) ± standard deviation as estimated from Namkoong (1979).
[c] Average across the four groups of the mean ± standard error of the mean among the four estimates.

a predictive index it might be necessary to test the given set of families in multiple testing periods, or to increase the number of replications in a given test. These are also the same five traits that were identified in the previous section as having higher family by test interaction; hence, this is likely why their heritabilities are lower.

Among the 16 traits evaluated, heritability estimates differed between at least two of the four groups for HEA, GAL, PGA, ADR, FR2, FAR, ROR, SHR, ENR, and RSS. Nevertheless, for these traits (except HEA, FR2 and ROR) the average family heritability across the four groups was higher than 0.70. The lower heritability estimate obtained for HEA (i.e., 0.23 for group 2) might be due to the lower incidence of healthy seedlings for families in this group (i.e., 0.03 on the average, ranging from zero to 0.10 across the 3 testing periods). This result is on the extreme portion of the binomial distribution, and even after transformation to a logistic scale, the ability to detect any variability among families is greatly reduced. For FR2 and ROR, family heritabilities were lower and less reliable (i.e., larger standard deviations) for groups 1 and 3, respectively, compared to the other groups, and on the average were also low (0.58 and 0.66 for FR2 and ROR, respectively). Thus, it is possible that these two traits (FR2 and ROR) are not under strong genetic control.

Genetic Correlations Among Traits

The first type of correlation discussed in this paper was for the same trait measured on individuals of the same genetic group but in different tests (i.e., r_g and r_g^*); this measures the repeatability of a given trait, as previously discussed. The second type was estimated between two different traits evaluated on the same individuals ($r_{g:i,j}$). This type of correlation, in some sense, is a measure of the commonness of the genes governing the determination of the two traits (Mode and Robinson, 1959).

In general, traits expressing initial infection (i.e., HEA, SYM, GAL, PGA, and ENR) and RSS (complex trait) were highly correlated among themselves (i.e., $r_{g:i,j} < -0.60$, or $r_{g:i,j} > 0.60$) (Table 2.8), but not strongly correlated with all other traits, except with SHR. White and Hodge (in preparation) suggested that the predictive value of an index of indirect observations could be improved by including in the index traits with a minimum amount of correlation, or by including a trait correlated with another trait in the index but not with the target trait. Therefore, any two traits expressing initial infection will likely be of minimum value in the same index, unless one of the traits is not correlated with field breeding values. Alternatively, promising indices to predict field breeding values could be constructed by combining one of the traits expressing initial infection with one trait expressing severity of infection, except SHR. The final decision about which traits yield a better index to predict field performance will depend on other parameters, as for example, family heritability, and genetic correlation with target trait being predicted.

The traits expressing severity of infection were also highly correlated among themselves (i.e., $r_{g:i,j} < -0.60$, or $r_{g:i,j} > 0.60$), except FR2 which was correlated only with FAR, THN, and RES, and the traits MED and ROR which were not correlated with any other trait. The trait MED, as discussed above, is not a well-defined trait and inclusion in an index is not recommended. However, the traits FR2 and ROR, even though they had low heritability estimates and measurable family x test interaction, may still be useful in an index to predict field breeding values, depending upon how they correlate with the field breeding values (target trait).

Table 2.8. Average genetic correlation ($r_{g(i,j)}$) over the four groups of 25 slash pine families and standard error of the mean in parentheses for all possible pairs of traits evaluated six months after artificial inoculation with fusiform rust fungus.

Traits[a]	SYM	GAL	PGA	PAD	ADR	FR2	FAR	THN	MED	ROR	SHR	TPR	ENR	RES	RSS
Health (HEA)[b]	0.62 (0.13)	-0.73 (0.13)	-0.79 (0.09)	-0.30 (0.16)	-.032 (0.19)	-0.19 (0.31)	-0.14 (0.27)	0.24 (0.20)	-0.04 (0.24)	-0.39 (0.25)	0.29 (0.15)	-0.19 (0.08)	0.81 (0.09)	0.11 (0.22)	0.73 (0.16)
Symno (SYM)[b]		-0.91 (0.02)	-0.96 (0.07)	-0.36 (0.16)	-0.19 (0.20)	-0.05 (0.20)	-0.24 (0.26)	0.47 (0.17)	-0.49 (0.16)	0.09 (0.18)	0.63 (0.24)	-0.46 (0.08)	0.93 (0.03)	0.32 (0.24)	0.95 (0.02)
No. galls (GAL)			0.93 (0.03)	0.36 (0.07)	0.22 (0.07)	0.27 (0.20)	0.21 (0.27)	-0.43 (0.17)	0.31 (0.16)	-0.03 (0.18)	-0.53 (0.08)	0.36 (0.24)	-0.98 (0.00)	-0.25 (0.24)	-0.93 (0.02)
Prop. of galls (PGA)[b]				0.37 (0.14)	0.23 (0.15)	-0.05 (0.34)	-0.05 (0.22)	-0.44 (0.20)	0.42 (0.18)	0.05 (0.15)	-0.57 (0.11)	0.44 (0.17)	-0.98 (0.00)	-0.28 (0.25)	-0.98 (0.03)
Prop. adv. shoots (PAD)[b]					0.98 (0.09)	0.17 (0.22)	0.46 (0.16)	-0.66 (0.18)	0.40 (0.15)	-0.39 (0.18)	-0.67 (0.09)	0.67 (0.22)	-0.34 (0.22)	-0.70 (0.25)	-0.37 (0.01)
No. adv. shoots (ADR)						0.98 (0.01)	-0.17 (0.14)	-0.47 (0.08)	-0.29 (0.20)	-0.15 (0.14)	-0.04 (0.21)	0.26 (0.15)	-0.21 (0.15)	-0.56 (0.06)	-0.21 (0.12)
Fat galls (FR2)[b]							0.83 (0.12)	-0.61 (0.25)	-0.41 (0.32)	-0.27 (0.14)	-0.69 (0.25)	0.62 (0.28)	-0.15 (0.27)	-0.70 (0.10)	-0.05 (0.23)
Gall size (FAR)								-0.89 (0.05)	-0.17 (0.17)	-0.04 (0.11)	-0.69 (0.12)	-0.83 (0.25)	-0.23 (0.28)	-0.89 (0.06)	-0.24 (0.23)
Thin galls (THN)[b]									0.06 (0.26)	0.39 (0.40)	0.88 (0.13)	0.34 (0.33)	0.41 (0.19)	0.94 (0.03)	0.47 (0.27)
Medium galls (MED)[b]										-0.42 (0.36)	-0.33 (0.13)	-0.37 (0.21)	-0.32 (0.19)	-0.06 (0.34)	-0.47 (0.18)
Rough galls (ROR)[b]											0.15 (0.21)	0.39 (0.24)	-0.04 (0.21)	0.33 (0.17)	0.07 (0.17)
Short galls (SHR)[b]												-0.90 (0.06)	0.52 (0.15)	0.88 (0.06)	0.58 (0.15)
Typical galls (TPR)[b]													-0.38 (0.20)	0.87 (0.06)	-0.45 (0.10)
Entry (ENR)														0.24 (0.260)	0.97 (0.01)
Post-entry (RES)															0.29 (0.25)

[a] For trait definitions see Table 2.2; [b] Binomial traits evaluated on logistic scale.

Conclusions

The results presented in this chapter suggest that 11 out of the 16 traits evaluated (i.e., SYM, GAL, PGA, and ENR, which are related to initial infection, and PAD, ADR, THN, SHR, TPR, and RES, which express severity of infection, and the complex trait RSS) are highly promising traits to be included in an index to predict field breeding values. For these traits, family x test interaction does not appear to be important, but standardization of the data may be necessary to achieve homogeneity of variances among testing periods. In general, differences in family variance, variance of family means, and heritability estimates among the four groups of families were only minor. On the average, these 11 traits had relatively high heritability estimates (i.e., $h_f^2 > 0.78$). The other 5 traits (i.e., HEA, FR2, FAR, MED, and ROR) had lower average heritability estimates (i.e., $h_f^2 < 0.70$), and also had a measurable amount of family x test interaction. These traits, except MED and HEA, are not strongly correlated with some of the most promising traits and thus, depending on their correlation with field results, may still be useful in an index to predict field breeding values.

CHAPTER 3
INDIRECT PREDICTION OF FIELD RUST BREEDING VALUES OF SLASH PINE PARENTS FROM OPEN-POLLINATED FAMILIES IN GREENHOUSE TESTS

Introduction

Fusiform rust caused by the fungus *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* is the most serious disease of slash pine (*Pinus elliottii* var. *elliottii*) and loblolly pine (*Pinus taeda* L.) in the southern U.S. Because of its severe effects on survival, growth and wood quality, a large portion of the breeding program conducted by the Cooperative Forest Genetics Research Program (CFGRP) is devoted to selection and breeding for resistance to fusiform rust. For the past 30 years, parental breeding values for rust resistance have been assessed by 1) growing offspring from selected parents in field progeny tests exposed to natural inoculum of the fungus, and 2) measuring the percent of individuals from each family that incurred the disease at 3, 5, and 10 years after planting. These field tests are relatively expensive, require 3 to 10 years for reliable results, and vary in precision with field conditions, especially with the amount of inoculum at the field site. Also, a field progeny test that is precise for assessing rust resistance requires relatively high incidence of disease (greater than 30%); but this level of infection may reduce the precision of predicting breeding values for other traits such as growth (Hodge and White, 1986) and wood properties.

Indirect testing procedures for fusiform rust, such as greenhouse screening of young seedlings after artificial inoculation, could conceivably increase precision

of predictions, decrease costs, and speed the time to obtain breeding value predictions. The breeding value predictions from such a procedure could be used 1) to screen wild selections prior to infusing them in the breeding population, 2) to retain only the best parents in the breeding population before crossing, 3) to eliminate some parents before field testing to reduce the size and costs of field tests, 4) to plan or rogue seed orchards at an early age to reduce the overall maintenance costs and increase gain from the orchard, and finally 5) to increase the precision of the predicted breeding values by combining field and greenhouse results.

An indirect testing technique for fusiform rust was first devised by Jewell (1960) and has been under constant improvement throughout the years (Miller, 1970; Powers et al., 1971; Laird and Phelps, 1975; Mathews et al., 1978; Anderson et al., 1983; Knighten et al., 1988). This technique has been used since 1970 at the Resistance Screening Center (RSC) established by the USDA Forest Service at Asheville, NC, to operationally screen large numbers of pine families for fusiform rust as a service for several organizations, researchers, and private industries (Anderson and Powers, 1985). Several studies have provided evidence that this early testing technique is feasible (Dinus, 1969; Jewell and Mallet, 1967; Wells and Dinus, 1974; Walkinshaw et al., 1980; Hubbard, 1981; and Skoller et al., 1985), and have identified several traits that, when combined in a selection index, might allow precise and consistent prediction of field breeding values (Walkinshaw et al., 1980). However, none of the preceding studies used a large enough sample of families to properly evaluate the efficiency of this indirect testing technique compared to field tests. In the first two chapters of this dissertation, 22 greenhouse traits were evaluated in three different testing periods for four groups of 25 families. Among these traits, 15 traits showed relatively high family heritability and low family x test interaction; thus, they may be useful in an index to predict field breeding values.

However, to efficiently use this indirect testing technique in the slash pine breeding program, several questions must be answered: 1) Which greenhouse traits should be measured?, 2) How should the information on the greenhouse traits be combined into a single prediction of field rust breeding values?, and 3) How efficient are the greenhouse tests (i.e., indirect tests) compared to field tests (i.e., direct tests)?.

If data are balanced and of equal quality for all candidates, then use of indirect traits to predict target traits (or any other type of correlated response) can be approached as an application of selection index. The theory of standard selection index is well described in the literature (Lin, 1978; Namkoong, 1979, ch. 4; Falconer, 1981, ch. 19; Bulmer, 1985, ch. 11; Bridgwater and Squillace, 1986; Van Vleck, 1988) and its application to indirect prediction in forest tree improvement is discussed by Burdon (1989) and White and Hodge (1989, chapters 9 and 10). In brief, a selection index combines information from several traits and/or sources into a single prediction of genetic value of a target trait. In theory, predictions of genetic value using an index selection are unbiased and have minimum error variance of prediction among all possible linear combinations of the data if 1) data are of equal quality and quantity for all candidates, and 2) the means, variances and covariances for all observations, and the covariances among observations of the indirect trait and the target trait genetic values are known. If in addition, the joint distribution of the indirect observations and field results are multivariate normal, then 1) selection index provides the best prediction among all possible functions and transformations of the data, 2) selection on the basis of the index maximizes the probability of selecting the better of any two candidates, and 3) the expected genetic progress is maximized for a fixed number of selections made (Henderson, 1963; White and Hodge, 1989).

The overall goal of this chapter was to apply selection index theory to predict breeding values for fusiform rust resistance in the field from measurements of one

or more greenhouse traits. The specific objectives were: 1) to determine which greenhouse traits should be combined in a selection index to allow prediction of field rust breeding values for populations with different mean levels of rust resistance (i.e., unimproved and improved), 2) to determine if multiple greenhouse tests for a given family materially improve predictions, 3) to compare newly developed selection indices to the index currently in use by the RSC, 4) to compare parental breeding values predicted from greenhouse data to those predicted from field tests, and 5) to explore when and how greenhouse tests for rust resistance (i.e., early testing and selection) should be used in the slash pine breeding program.

Materials and Methods

Plant Material

Four groups of 25 open-pollinated families each were obtained from two different populations of slash pine parents in the Florida Cooperative Forest Genetics Research Program (CFGRP). The complete description of the selection procedures and characteristics of the two populations are presented in chapter 2. In brief, families in groups 1, 2, and 3 were randomly chosen from a population unimproved for rust, while families in group 4 were chosen from a population previously selected for rust resistance (i.e., an improved population). The parents within each group were evaluated in at least four field progeny tests, and were in the same first-generation clonal orchard to minimize differences in maternal effects and pollen background within a group (Table 3.1).

The breeding values for rust resistance (R50) for these parents were predicted from data from four to 20 open-pollinated field progeny tests using best linear prediction (White and Hodge, 1988). An R50 expresses the expected percent of offspring from a given parent (assuming it was mated with other parents with the

Table 3.1. Characteristics of the four groups of 25 slash pine parents and the number of field tests in which all families are contained.

Groups	Pop.[a]	Mean R50[b]	Field Tests[c] Number	Field Tests[c] Ages[d]	Field Tests[c] Total[e]
1	1	39.5 ± 20.0	4	3, 5	6
2	1	49.1 ± 19.8	5	3, 5	8
3	1	51.9 ± 18.5	4	3, 5, 10	7
4	2	31.7 ± 17.3	6	3, 5, 10	11

[a] Population 1 contains 1397 parents unselected for rust with mean R50 of 50 and standard deviation of 22. Population 2 is a seed collection area in Wayne, GA, containing 384 parents selected for freedom from rust in a highly infected stand; population 2 has mean R50 of 36 and standard deviation of 14.

[b] R50, the predicted parental breeding values for fusiform rust resistance were predicted from all data available for each parent (i.e., 4 to 20 field tests) and not just from these field tests.

[c] These are field tests which contain all parents in each group. Most parents are also represented in other field tests that do not contain the complete set of families. For detailed information about each field test see Appendix 3.

[d] Ages at which field incidence of rust (1=presence, 0=absence) were assessed for some field tests in each group.

[e] Total number of field assessments for each group.

same average R50) that will be infected on a field site in which average material experiences 50% infection. The estimated correlations between the true and predicted predicted breeding values (an estimate of precision) using all 2,245 parents in the CFGRP are 0.79 and 0.84 for parents in 3 to 5, and 5 or more tests, respectively; thus, these predictions of field breeding values are fairly precise (White and Hodge, in preparation).

Greenhouse Tests

The experiment was conducted at the RSC using their standard concentrated basidiospore suspension technique (Anderson et al., 1983; Knighten et al., 1988). In brief, the procedures were: seed stratification; seed sowing in 10 x 15 x 5 cm germination trays in the greenhouse; transplanting germinants into Ray Leach tubes (three weeks after sowing); seedling inoculation (five weeks after transplanting) with a basidiospore suspension (20,000 spores per ml) composited from spores collected in five slash pine growing areas; incubation in a chamber for 24 hours (20.5°C and 97% humidity); and incubation in greenhouse (20°C and 12hr photoperiod) until evaluation six months after inoculation.

Each group of 25 families was tested three times during the year (i.e., winter, summer and fall inoculations). To maintain standard greenhouse conditions (20°C and 12 hr photoperiod), artificial light, heat and cooling were supplied as required. The description of the environmental conditions during each testing period is presented in chapter 1. For each group in each test, 120 seedlings of each family were inoculated. The 120 seedlings were divided into six sets of 20 seedlings each (a plot), and were inoculated in two runs (3 sets each) one day apart. The inoculated seedlings were placed in a humid chamber for 24hr and then were randomly placed in the greenhouse in six blocks (1 set per block and 3 blocks per run). Thus, a total of 3,000 seedlings (20 seedlings x 25 families x 2 runs x 3 blocks) were inoculated in each test for each group, and a total of 36,000 seedlings were used in the entire experiment (3,000 seedlings x 4 groups of 25 families x 3 testing periods).

Six months after inoculation the following traits were assessed: presence (1) or absence (0) of ten different fusiform rust symptoms, the number of adventitious shoots present in the galled area, and the number of galls per seedling. Several other traits (i.e., FAR, ENR, RES and RSS) were constructed by combining several

individual traits. Traits specifying gall characteristics were evaluated on the most prominent gall on each seedling (the normal RSC procedure) and on all galls present on each seedling. A total of 22 different traits were evaluated, but only the 15 most promising traits as suggested in the previous chapters are considered here (Table 3.2). The binomial traits (SYM, HEA, PGA, PAD, FR2, THN, ROR, SHR, and TPR) were analyzed after logistic transformation of the plot means as suggested in chapter 1.

Field Tests

An inventory of the CFGRP database showed that from four to seven field tests were available that contained all families in a given group (Table 3.1). Families from different groups were always tested in different tests. The field tests are all randomized complete block designs with several members of each family in each block planted in row plots. The number of trees per plot, and the number of blocks, varied from test to test. The incidence of rust was evaluated at ages 3, 5, and 10, but not all tests were evaluated at all three ages. This data set was used to estimate correlations between field incidence of rust and greenhouse traits, and also to compare the efficiency of greenhouse versus field tests.

Estimating Index Coefficients

To develop one or more selection indices that are predictive of field rust resistance involves 1) choosing the appropriate traits (out of 15 measured), and 2) developing a set of coefficients that weights the greenhouse traits according to their value in predicting field breeding values. The breeding value predictions ($\hat{g}$) are parental breeding values and their predictions are based on the assumption that all material sent to the RSC will be unrelated open-pollinated families (i.e., half-sib families).

Table 3.2. Name, abbreviation and description of the different traits evaluated in greenhouse tests six months after inoculation of slash pine seedlots with fusiform rust fungus.

Name	Abbreviation	Description
Healthy	HEA[a]	Seedling showing no rust symptoms.
Symno	SYM[a]	Seedling showing area of purplish discoloration and/or needle base with purple discoloration.
Gall	PGA[a]	Seedling with swellings or bumps on the stem.
	GAL	Number of galls present on seedling.
Adventitious Shoots	ADR	Number of adventitious shoots emerging at acute angles along the galled portion of the stem.
	PAD[a]	Galled seedling with presence of adventitious shoots.
Fat Gall	FR2[a]	Gall that is twice the diameter of the stem if it were not galled.
Gall Size	FAR	Gall classified as: 1 fat, 0.5 medium, and 0 thin.
Thin Gall	THN[a]	Gall that is 1 1/3 or less than the diameter of the stem if it were not galled.
Rough Gall	ROR[a]	Gall with an area of discoloration covering more than 50% of the gall surface making it rough.
Short Gall	SHR[a]	Gall less than 25mm in length.
Typical Gall	TPR[a]	Gall with swelling around the entire circumference of the stem.
Entry	ENR	Seedling classified as: 2.5 healthy, 2 symno, 1 one gall, 0.5 two galls, and 0 more than 2 galls.
Post-Entry	RES	Seedlings whose values is the sum of the following scores: 0.5 if no adventitious shoots, 1 if gall is short, 1 if gall is not fat, and 1 if gall is atypical. Ranges of values is 0 to 3.5 per seedling.
RSS	RSS	Seedling classified as: 0 non-rough, long gall; 0.25 non-rough, short gall; 0.5 rough, long gall; 1 rough, short gall; 2 symno only; and 3 healthy seedling.

[a] Binomial traits evaluated as presence (1) or absence (0) of the symptom. Plot means of all binomial traits were transformed to the logistic scale for all analyses.

The general form of a selection index (boldface type indicating vectors and matrices) can be written as:

$$\hat{g} = \mathbf{b}'(\mathbf{y} - \alpha)$$

where

$\hat{g}$, a scalar, is the predicted breeding value for fusiform rust (in percent) as it would be expressed under field conditions,

$\mathbf{b}' = \mathbf{c}'\mathbf{V}^{-1}$, is a 1 x n vector of index coefficients,

$\mathbf{y}$ is an n x 1 vector of observed family means for n greenhouse traits from a given test,

α is an n x 1 vector of expected values (i.e., means) of the observed family means for n greenhouse traits from a given test,

$\mathbf{c}'$ is 1 x n vector containing covariances between the family means in $\mathbf{y}$ and the breeding values being predicted (i.e, the incidence of rust under field conditions),

$\mathbf{V}$ is an n x n matrix of variances and covariances of family means among the greenhouse traits.

The results in chapter 2 indicated that variances among family means from greenhouse data changed from test to test. Hence, standardization of the data will allow development of index equations with constant coefficients, that can be applied to all RSC tests in the future. The data vector $\mathbf{y}$ contains family means for each trait averaged over 120 seedlings (i.e., 20 seedlings per plot x 3 blocks x 2 runs), and α contains the mean of all family means in a given test for each trait. Then, each element of $(\mathbf{y} - \alpha)$ is divided by the estimated standard deviation of family means for each trait and test; these observations are standardized family means.

To determine if different indices are needed for the unimproved versus improved populations, three types of indices were constructed: type A, using information from all four groups of families (i.e., unimproved and improved populations); type B, using information only from the unimproved population (i.e.,

groups 1, 2, and 3); and type C, using information only from the improved population (i.e., group 4). For each type of index, index coefficients were estimated for all possible combinations of n = 1, 2, 3, 4, 5, and 6 greenhouse traits. This was done both for family means averaged over one and two tests to examine the benefits of screening families for fusiform rust in more than one greenhouse testing period.

Estimating the Elements of **V** and **c**.

The **V** matrix and the **c** vector apply to the standardized observations; thus **V** is a correlation matrix with ones on the diagonal ($\text{Var}(\overline{y}) = 1$ for all traits), and correlations of family means (standardized covariances) on the off-diagonal. To estimate the elements of **V**, analyses of covariance were conducted on standardized observations for all traits, pooled across three testing periods as follows: 1) for type A indices using information of all four groups of families (Table 3.3), 2) for type B indices using information from the three groups from the unimproved population (i.e., groups 1, 2, and 3) (Table 3.3), and 3) for type C indices using information from group 4 (i.e., from the improved population) (Table 2.3, chapter 2).

From the above analyses of covariance, the covariance of family means was estimated between all pairs of traits as follows:

$\text{cov}(\overline{y}_i, \overline{y}_j) = \sigma_{fg:i,j} + \sigma_{ftg:i,j}/t + \sigma_{e:i,j}/nt$, for the analysis of covariance pooled across four and three groups families (Table 3.3), and

$\text{cov}(\overline{y}_i, \overline{y}_j) = \sigma_{ft:i,j} + \sigma_{ft:i,j}/t + \sigma_{frt:i,j}/rt + \sigma_{e:i,j}/nrt$, for the analysis of covariance for group 4 (Table 2.3, chapter 2),

where

$\sigma_{fg:i,j}$ is the covariance component between traits i and j associated with family effects within a given group,

$\sigma_{ftg:i,j}$ is the covariance component between traits i and j associated with family

by test interaction within a given group effects,

$\sigma_{ft:ij}$ is the covariance component between traits i and j associated with family effects,

$\sigma_{ft:ij}$ is the covariance component between traits i and j associated with family by test interaction effects,

$\sigma_{frt:ij}$ is the covariance component between traits i and j associated with family by run interaction nested in test effects,

$\sigma_{e:ij}$ is the covariance component between traits i and j associated with plot effects,

t = number of tests (t = 3), r= number of runs (r = 2), b = number of blocks (b = 3), and n = number of replications (i.e., pooled blocks and runs).

The elements of **c** are covariances between the standardized family means for the greenhouse traits (in **y**) and the breeding value which is being predicted (**g**). We wished to predict breeding values for rust resistance (in percent) for a 50% hazard site. This was done in two steps. First, we calculated the family mean correlations between greenhouse traits and field incidence of disease for each pair of greenhouse and field tests. The number of correlations estimated for each trait was 24, 24, 21, and 33 for groups 1, 2, 3, and 4, respectively. Second, we converted the correlations into covariances using the variances of family means for greenhouse and field traits. For some of the greenhouse traits, the family mean correlations between greenhouse and field traits varied with the percent of rust in the field test. Since we wished to predict breeding values for a 50% hazard field site, linear regression models predicting correlations as a function of the percent of rust in the field test and the percent of rust squared were developed for all traits combined across 4 and 3 groups of families. Among the 15 greenhouse traits evaluated, the correlation models were significant at p = 0.01 for the traits HEA, SYM, GAL, PGA, ENR, and RSS (Table 3.4). For these five traits, the models were used to

Table 3.3. Analysis of covariance and expected mean cross products for any two fusiform rust traits combined across either four groups of families or across three groups of 25 slash pine families.

Source of Variation	DF1[a]	DF2[b]	Expected Mean Cross Products[c]
Groups	3	2	$\sigma + n\sigma_{flg} + nt\sigma_{fg} + k\sigma_{btg} + nk\sigma_{gt} + tk\sigma_{bg} + ntk\sigma_g$
Tests	2	2	$\sigma + n\sigma_{flg} + k\sigma_{btg} + nk\sigma_{gt} + nkl\sigma_t$
Replications(Group)[d]	20	15	$\sigma + k\sigma_{btg} + tk\sigma_{bg}$
Group x Test	6	4	$\sigma + n\sigma_{flg} + k\sigma_{btg} + nk\sigma_{gt}$
Rep x Test(Group)	40	30	$\sigma + k\sigma_{btg}$
Family(Group)	92	68	$\sigma + n\sigma_{flg} + tn\sigma_{fg}$
Family x Test(Group)	184	136	$\sigma + n\sigma_{flg}$
Error	1379	1020	σ_e
Total	1726	1277	-

[a] Degrees of freedom for analysis combined for groups 1, 2, 3, and 4.
[b] Degrees of freedom for analysis combined for groups 1, 2, and 3.
[c] For analyses combined over groups 1, 2, 3, and 4, n = 6, t = 3, k = 25, and l = 4.
  For analyses combined over groups 1, 2, and 3, n = 6, t = 3, k = 25, and l = 3.
[d] Block and runs were pooled (i.e., replications) to make the analysis tractable computationally.

predict the mean correlation between greenhouse traits and field rust infection in a 50% hazard environment. For the greenhouse traits with non-significant models, the average correlation over all observations was used. For the improved group (i.e., group 4), the number of estimated family mean correlations was not large enough to develop models. Thus, the average correlation over testing periods and field tests was used for each greenhouse traits.

Table 3.4. Predictive equations for family mean correlations between incidence of fusiform rust in the field (x) and the six greenhouse traits with significant correlation models.

Trait[a]	Population[b]	Linear Model	P-value[c]	R^2
HEA	1,2	$0.3696 - 2.0762(X) - 1.7539(X^2)$	0.000	0.18
	1	$0.3560 - 2.0652(X) - 1.7949(X^2)$	0.006	0.16
SYM	1,2	$0.3360 - 2.2868(X) - 1.7964(X^2)$	0.000	0.22
	1	$0.1099 - 1.7585(X) - 1.5289(X^2)$	0.009	0.14
GAL	1,2	$-0.4858 + 3.0517(X) - 2.7569(X^2)$	0.000	0.22
	1	$-0.2846 + 2.4808(X) - 2.4096(X^2)$	0.001	0.20
PGA	1,2	$-0.3909 + 2.4465(X) - 1.9137(X^2)$	0.000	0.26
	1	$-0.1867 + 1.9826(X) - 1.6943(X^2)$	0.003	0.18
ENR	1,2	$0.4639 - 2.8404(X) + 2.4466(X^2)$	0.000	0.24
	1	$0.2747 - 2.3270(X) + 2.1507(X^2)$	0.001	0.20
RSS	1,2	$0.4429 - 2.6637(X) + 2.0583(X^2)$	0.000	0.31
	1	$0.2985 - 2.4001(X) + 1.9938(X^2)$	0.000	0.25

[a] For trait definitions see Table 3.2.
[b] Population 1 and 2 are unimproved and improved for fusiform rust, respectively. The number of observations used to build each equation is 63 and 96 for population 1 and populations 1 and 2 combined, respectively.
[c] Significance level of the entire model. Also, all variables in each model are significant at P=0.05.

The family mean correlation for each trait (Appendix 4) was then converted into a covariance (the elements of **c**) following procedure outlined by White and Hodge (1989). In this case the equation reduced to:

$$c_{i1} = 2 \times r_{FG:ij} \times (Var\ (\overline{y}_F))^{0.5}$$

where

c_{i1} is the family mean covariance between greenhouse trait, i, and the incidence of rust in a 50% rust hazard environment,

$r_{FG:ij}$ is family mean correlation between greenhouse trait, i, and the incidence of

rust in a 50% rust hazard environment,

Var $(\overline{y}_F)$ = Variance of family means in field test with 50% rust, estimated using models developed from the CFGRP data set and equal to 0.01835 and 0.02184 for improved and unimproved populations, respectively (White and Hodge, 1989). Note that the variance of family means for greenhouse traits equals one because of standardization of the greenhouse data, and the right side is multiplied by 2 because parental breeding values are being predicted from half-sib family data.

Selecting the Indices

The goal was to identify one or two promising indices to predict field rust breeding values assuming greenhouse data for each family are available for either one or two greenhouse tests. Indices were evaluated by 1) the estimated correlation between the predicted and true breeding values, 2) the biological interpretation of the index (i.e, type of traits and the signs of their coefficients), 3) the correlation between the predicted breeding values based on greenhouse data and the R50's predicted from field data, and 4) efficiency of selection at different truncation points. First, for each type of index (i.e., type A, B, and C), for both one and two greenhouse tests, the best 50 indices (i.e., equations) for 2, 3, 4, 5, 6 traits, and 5 indices with one trait, which had the highest correlation between the true and predicted breeding values were selected. This correlation measures the precision of the index, and is calculated the following procedure outlined by White and Hodge (1989) as corr$(\hat{g}, g)$ = $[\mathbf{c'V^{-1}c} / \sigma_A^2]^{0.5}$, where σ_A^2 is the predicted additive genetic variance of the target trait, and is equal to 0.04874 and 0.06222 for the improved and unimproved populations, respectively. The σ_A^2 predictions were obtained using models developed from 28 open-pollinated progeny tests (White and Hodge, 1989).

Second, any equation selected in step 1, with a coefficient on a trait opposite to the sign of the genetic correlation between greenhouse trait and field percent of infection was eliminated from further considerations. Next, the best 39 equations for use with data from families screened in one greenhouse test and 32 equations for data from two greenhouse tests were further examined by comparison with predicted parental breeding values from field data (R50). The correlation between the predicted breeding values from greenhouse data and the R50's (corr($\hat{g}$, R50)), and the efficiency of selecting the top and bottom 25%, and at 50% selection intensity were calculated for all selected equations for each group and greenhouse testing period. To estimate the correlation between predicted breeding values and R50's, families were paired and correlations calculated between predicted breeding values from greenhouse data ($\hat{g}$) and those predicted from field data (R50). The R50's are quite precise, that is they are believed to be close to the true breeding values. In this case, these correlations should be close to the correlations between the true and predicted breeding values calculated as $(\mathbf{C'V^{-1}C}/\sigma_A^2)^{0.5}$.

A measure of the efficiency of selection was used to compare the genetic gain from selection based on the greenhouse index to the genetic gain possible from selection based on 'true' genetic values (i.e., R50) and was calculated as

Efficiency = (R50* - R50)/(R50** - R50)

where

R50* is the mean R50 for the families selected based on the greenhouse index,

R50** is the mean R50 for the families selected based on the R50's, and

R50 is the overall mean R50 for the given group of families.

For each of the 71 selected equations, the mean corr($\hat{g}$, R50) and the mean efficiency of selection at 25 (top and bottom) and 50% selection were examined after averaging over the three testing periods and over all four groups, over the three groups, and for the resistant group. Finally, one or two indices was selected

for use with data from one and two greenhouse tests on the basis of high corr($\hat{g}$,R50), high efficiency of selection at 25 (top and bottom) and at 50% selection intensity, and stable performance across all groups and testing periods (i.e., small standard deviations for efficiency estimates).

Results and Discussion

Constructing and Testing Indices

To determine if a different index was needed for each population (unimproved and improved), indices were developed using parameters estimated 1) from data on all four groups of families (type A index), 2) from data on the three groups of families from the unimproved population (type B index), and 3) from data on the group of families from the population improved for fusiform rust resistance (type C index). For each type of index, and for data from either one or two greenhouse tests, 9,948 equations were constructed (total of 56,688 equations). Among the 9,948 equations developed for each index type, and for either one or two greenhouse tests, 50 equations with 2, 3, 4, 5, and 6 traits, and 5 equations with one trait, with the highest estimated correlation between the true and predicted breeding values (corr($\hat{g}$, g)) were selected. For indices with more than two traits, corr($\hat{g}$, g) was very similar among the 50 selected equations for a given number of traits, and not much different among equations with different number of traits. Some equations were eliminated because of the sign on the coefficient, or because of the combination of traits in the equation, as discussed above. Across all types of indices, 39 and 32 biologically-sound indices, for one and two greenhouse tests, respectively, were chosen for further examination. For each one of these indices, the correlation between the predicted breeding values and field R50's (corr($\hat{g}$, R50)), and the efficiencies of selection at 25% and 50% selection intensity, were

estimated for each of the four groups of families and for each greenhouse test. The estimated correlations and efficiency of selection for each index were then averaged over all greenhouse testing periods across 1) the four groups of families, 2) the three groups of families from the unimproved population, and 3) the improved population. None of the 39 indices for one greenhouse test nor the 32 indices for two greenhouse tests was the best index for both unimproved and improved populations. Therefore, we selected one index for each population (unimproved and improved) that had 1) high average corr($\hat{g}$, R50), 2) high efficiencies of selection at 25 and 50% selection intensity, and 3) had stable predictions across groups and tests (i.e., with small standard deviations of the estimates). It is interesting that even though the choices of the best index to use given data from one or two greenhouse tests were made independently, the selected indices for one and two greenhouse tests contain the same set of traits for both the unimproved and improved populations (Table 3.5). Thus, any time that information from a second test for a given family is available, the predicted breeding value can easily be recalculated. In addition, all traits in the indices have high family heritabilities (i.e., $h_f^2 > 0.70$, from chapter 2), but some traits in the same index have high genetic correlations, as for example, SYM and GAL in U1 and U2 ($r_{g(i,j)} = -0.91$). We allowed such correlated traits in the same index because one of the traits might be adjusting the information provided by the other trait. For example, suppose that two families have the same incidence of SYM, but different numbers of galls (GAL) per galled seedling. The family with fewer galls per seedling may be more resistant; therefore, by using both traits in the same index, the two families will have different predicted breeding values, that may be closer to the true breeding values.

To compare the selected indices (U1, U2, R1, and R2) to the index currently used by the RSC (Hubbard, 1981), the correlation between the RSC predictions and R50's, and the efficiencies of selection at 25% and 50% selection intensity based

Table 3.5. Selection indices to predict field fusiform rust breeding values ($\hat{g}$) from indirect testing under greenhouse conditions for slash pine populations with different levels of rust resistance

ID[a]	Index	Use[c]
U1	$\hat{g}$[b] = - 0.0335(SYM) + 0.06201(GAL) + 0.03432(PAD) + 0.01862(FAR) - 0.0323(SHR)	Unimproved population, one test
U2	$\hat{g}$ = - 0.0283(SYM) + 0.07323(GAL) + 0.04063(PAD) + 0.02806(FAR) - 0.0355(SHR)	Unimproved population, two tests
R1	$\hat{g}$ = -0.14 + 0.0471(GAL) + 0.0544(FAR) - 0.0366(SHR)	Improved population, one test
R2	$\hat{g}$ = -0.14 + 0.0361(GAL) + 0.0480(FAR) - 0.0738(SHR)	Improved population, two tests

[a] ID is identification.
[b] $\hat{g}$ is the predicted breeding values for rust resistance from indirect testing under greenhouse conditions.
[c] Note that the same traits but different coefficients should be used when a given set of families are tested once or twice in the greenhouse.

on the RSC index were calculated for both unimproved and improved populations. The newly predicted breeding values were more correlated with field-predicted breeding values (i.e., R50) than RSC predictions. Such correlations assess fit over the entire distribution, and depending upon the use of the index, we may be interested in different portions of the distribution. Hence, we examined the efficiency of selection at different portions of the distribution. The efficiency of selection for the new indices, averaged over 9 tests for the unimproved population (i.e., 3 groups x 3 testing periods) and over 3 tests for the improved population, were higher and more stable than for the RSC index in all criteria (Table 3.6). Even the index developed on data from the improved population (i.e., R1 and R2) were more efficient when applied to the unimproved population than the RSC index either to eliminate the worse 25% or to select 50% of the population. These indices (i.e., R1 and R2) were developed with no data in common with the unimproved population, and still proved to be more efficient than the RSC index; this is strong proof of the adequacy of the selection index approach compared to the RSC index. Another interesting difference between the new indices and the RSC index occurred for the estimated variance among predictions (results not shown). According to White and Hodge (1989) for selection index approach, the predictions tend to spread out more (i.e., have larger variance among predictions) when they are based on a large quantity and higher quality data. Indeed, for the new indices (i.e., U1 and R1 compared to U2 and R2, respectively), the variances among prediction assuming two greenhouse tests were larger than for one greenhouse test. In contrast, for the RSC index, the variance among predictions from one test was larger than from two tests.

Another advantage of the newly developed indices is the interpretation of the predictions. That is, the breeding values predicted by the new indices express the expected percent of open-pollinated offspring of a given parent that will be infected

Table 3.6. Estimated correlations between true and predicted breeding values for fusiform rust resistance, and efficiencies of selection for different indices for slash pine populations with different levels of resistance.

Population Type	Index[c]	Corr $(\hat{g}, R50)$	Efficiency of Selection		
			Top25 ± STD	Bot25 ± STD	M50 ± STD
Unimproved[a]	RSC	-0.44	0.44 ±0.13	0.34 ±0.42	0.40 ±0.23
One test	U1	0.54	0.51 ±0.16	0.54 ±0.14	0.52 ±0.22
	R1	0.49	0.47 ±0.15	0.47 ±0.37	0.48 ±0.20
Unimproved	RSC	-0.47	0.46 ±0.20	0.39 ±0.34	0.37 ±0.28
Two tests	U2	0.57	0.54 ±0.22	0.56 ±0.14	0.58 ±0.17
	R2	0.51	0.49 ±0.18	0.49 ±0.18	0.64 ±0.14
Improved[b]	RSC	-0.25	0.31 ±0.02	0.01 ±0.25	0.20 ±0.07
One test	U1	0.36	0.26 ±0.16	0.48 ±0.11	0.34 ±0.03
	R1	0.49	0.45 ±0.14	0.54 ±0.09	0.31 ±0.03
Improved	RSC	-0.28	0.44 ±0.08	0.02 ±0.16	0.31 ±0.04
Two tests	U2	0.41	0.43 ±0.10	0.51 ±0.08	0.31 ±0.02
	R2	0.55	0.45 ±0.08	0.70 ±0.21	0.47 ±0.05

[a] The correlation and efficiency estimates for the unimproved population were averaged across groups 1, 2, and 3 and testing periods.
[b] The correlation and efficiency estimates for the improved population were averaged across 3 testing periods for group 4 (see Table 3.1).
[c] RSC index was developed by Hubbard (1981) and has since then been used by the Resistance Screening Center. The predictions for RSC index for two tests were obtained by averaging the index values over two tests. U1, U2, R1 and R2 are in Table 3.5.

on a field site in which average material experiences 50% infection. In contrast, predicted values from the RSC index can not easily be translated into a measure of resistance in the field (see predicted values in Appendices 5 and 6). Also, because the predicted values from the new indices (i.e., U1, U2, R1, and R2) are estimates of parental genetic values, expected gains from selection can be calculated

by averaging the index values of the selected individuals. For example, suppose that group 1 has an average index value of 50%, and a subset (say 1/4) of the parents with highest index values are selected (e.g., average selected = 31%), then the average index value for the selected parents minus the overall index average (e.g., -19%) expresses the reduction in rust incidence expected when offspring of the selected parents are planted in a 50% hazard site.

Even though we assumed that a single set of coefficients was appropriate for all families from a given population, these coefficients can be revised as new data either from research or commercial tests become available (i.e., better estimates of V and c); thus, possible selection errors made using the previous index can be corrected. Suppose, that new data shows that the family mean variances and covariances estimated for FAR were too small; then if we construct a new V, and recalculate the index, a different sets of weights will be generated. By applying this updated index to the old data we can upgrade the predictions.

Operational Implementation of the New Indices

Families tested in future greenhouse tests probably will not have been tested in the field, so we will not be able to develop a new index for each test. Hence, the indices in Table 3.5 should be used. One major issue in the implementation of the new indices is the decision of which index to use. The U1 and U2 indices are recommended for families from unimproved populations (i.e., not previously selected for rust resistance), and R1 and R2 for families from improved populations. For a population with unknown level of resistance (i.e., no prior information available), one approach is to use the U1 index. This index may not be as good as R1 if the population is resistant, but is still better than the RSC index, especially to eliminate the bottom 25% of the families. If the population is unimproved, the U1 index is more efficient in selection, and more reliable (smaller standard deviation) than R1.

Alternatively, by comparing the test mean with some function of the resistant and susceptible seedlots, it may be possible to determine whether the material being tested is unimproved or improved for fusiform rust resistance. These seedlots have been used for several years by the RSC as standard checklots to compare resistant and susceptible families. Models to predict the test mean for the unimproved and improved populations as functions of the susceptible and resistant seedlots were developed for all traits in U1 and R1 and for PGA (Table 3.7). For example, suppose that the test means for PAD in a new test, after transformation to a logistic scale, are 0.721, 1.257 and 0.169 for all families, susceptible and resistant seedlots, respectively. Using the models in Table 3.7, we predict that the test mean over all families is 0.737 and 0.116 for an unimproved and improved population, respectively. The actual test mean for PAD (0.721 in this example) is closer to the predicted test mean for unimproved material. This, indicates that the families being tested are likely unimproved for rust resistance; therefore, the U1 index should be used to predict field breeding values for these families. Models for other traits, especially for PGA and GAL, should be evaluated in deciding which index to use.

Since the decision of which index to use will not be made until after we evaluate the test, five different traits (i.e., SYM, GAL, PAD, FAR, and SHR) need to be evaluated. In addition, the number of galled seedlings and the number seedlings alive at the evaluation time need to be recorded. The traits are evaluated on a seedling basis but may be recorded as total per plot. The binomial traits (SYM, PAD, and SHR) are transformed to a logistic scale following the procedure outlined by Cox (1969) as $Y_j = \log [(R_j + 0.5) / (n_j - R_j + 0.5)]$. Where, Y_j is the trait j expressed on logistic scale, R_j is the total incidence of jth trait in a given plot, and n_j is the total number of seedlings per plot for SYM, and the total number of galled seedling per plot for PAD and SHR.

Table 3.7. Prediction equations for test means (α) as a function of resistant (RES) and susceptible (SUS) seedlot means for several greenhouse traits when a small number of families are screened for fusiform rust resistance under greenhouse conditions at the RSC.

Trait	Intercept[b] 1	2	Model[c]	P-Value[d]	R^2
SYM[a]	-2.2765	-1.3666	0.3689(RES)	0.002	0.46
PGA[a]	1.9330	1.3426	-1.3449(RES) - 0.1637(SUS) + 0.7699(RES*SUS)	0.000	0.66
GAL	-1.1701	-1.3548	1.5561(RES) + 1.8782(SUS) - 1.2376(RES*SUS)	0.000	0.89
PAD[a]	0.1079	-0.5127	-0.5630(RES) + 0.4739(SUS) + 0.6060(RES*SUS)	0.000	0.93
FAR	0.1792	0.1983	0.1045(RES) + 0.5462(SUS)	0.001	0.58
SHR[a]	0.6393	1.9332	1.0390(SUS)	0.002	0.44

[a] Binomial traits evaluated on logistic scale.
[b] Use intercept 1 and 2 for predicting test means for unimproved and improved populations, respectively.
[c] RES and SUS are test means for the resistant and susceptible seedlots for each trait, respectively.
[d] Significance level of the model. Also, all variables in each model are significant at P=0.05.

After measurements are completed, plot means should be averaged to obtain family means for the jth trait. For all traits, the family means should be standardized as $Z = (\overline{y} - \alpha)/Var(\overline{y})$. When a large number of families are tested (more than 20) both the variance of family means ($Var(\overline{y})$) and α (the overall test mean) can be estimated directly from the data. However, when a small number or non-random sample of families is tested, the estimated variance and test mean from the data may not be appropriate. In this case, one alternative is to use the variance of family means and test means estimated from this study (Table 3.8). Another approach would be to estimate α as function of the susceptible and resistant checklots using the models on Table 3.7. These alternative approaches for small test are suggestions, and should be evaluated before application.

Table 3.8. Overall test means, checklot means, and variance of family means (V(y)) for unimproved and improved populations of slash pine families six months after artificial inoculation with fusiform rust fungus.

Trait	Pop.[b]	Test Mean	Checklots Means		$V(\overline{y}_1)$[c]	$V(\overline{y}_2)$[d]
			Resistant	Susceptible		
SYM[a]	1	-2.479	-1.390	-2.783	0.361	0.359
	2	-1.911	-1.390	-2.783	1.036	0.948
PGA[a]	1	1.979	0.789	2.328	0.397	0.347
	2	1.229	0.789	2.328	1.077	1.003
GAL	1	1.151	0.859	1.223	0.029	0.025
	2	0.948	0.859	1.223	0.074	0.069
PAD[a]	1	0.891	0.169	1.257	0.241	0.191
	2	0.445	0.169	1.257	0.146	0.102
FAR	1	0.581	0.467	0.660	0.005	0.004
	2	0.630	0.467	0.660	0.009	0.008
SHR[a]	1	-1.813	-1.224	-2.758	0.296	0.251
	2	-0.940	-1.224	-2.758	0.186	0.123

[a] Binomial traits on logistic scale.
[b] Populations 1 and 2 are unimproved and improved for fusiform rust, respectively.
[c] Variance of family means for unimproved and improved populations, assuming one greenhouse tests. Note, the variance of family means for the unimproved populations was averaged over 3 groups of families and 3 testing periods.
[d] Variance of family means for unimproved and improved population, assuming two greenhouse test. Note, the variance of family means for the unimproved population was averaged over 3 groups of families and 3 testing periods.

Efficiency of Greenhouse Tests Compared to Field Tests

Even though the new indices (i.e., U1, U2, R1, and R2) are better than the RSC index, the use of the greenhouse test as the only test procedure to evaluate resistance to fusiform rust in the slash pine breeding program depends on how efficient this indirect testing procedure is compared to the direct field tests. To

compare how the two testing techniques (i.e., field vs. greenhouse) rank the same set of parents, the Spearman's rank correlations between field predicted R50's and 1) family mean percent of rust for the field tests, and 2) the predicted breeding values from U1 and R1, were calculated for 23 and 11 field tests, and for 9 and 3 greenhouse tests for the unimproved and improved populations, respectively. On the average, family means from a single field test had higher rank correlations with R50's for both the unimproved (0.57) and improved populations (0.55), than either U1 rankings for the unimproved population (0.47) or R1 rankings for the improved population (0.36). Even though these correlations on the average were higher for field tests, family ranks for some field tests were less correlated with R50 then were the family ranks based on greenhouse tests. The correlation between family ranks from a single field test and R50's ranged from 0.12 to 0.90, and from 0.38 to 0.78, for unimproved and improved populations, respectively. In contrast, the correlation between family ranks based on greenhouse predictions and R50's, were more stable, ranging from 0.20 to 0.80, and from 0.30 to 0.40 for unimproved and improved populations, respectively.

Comparisons were also made between greenhouse and field tests for efficiency of selection at 25% and 50% selection intensity (Figure 3.1). For the unimproved and improved populations, selection based on family means for rust infection from a single field test was more efficient than selection based on greenhouse testing. Nonetheless, the differences in selection efficiency were not large enough to rule out greenhouse testing as an alternative or additional testing technique. This early testing and selection procedure may not be as good as we wish it to be, but field tests are not without problems. For example, for reliable differentiation among families for rust resistance from field testing, moderately heavy infection is required (Sohn et al., 1975; Schmidt and Goddard, 1971). This condition, however, is not always found in field tests, and even in high hazard areas,

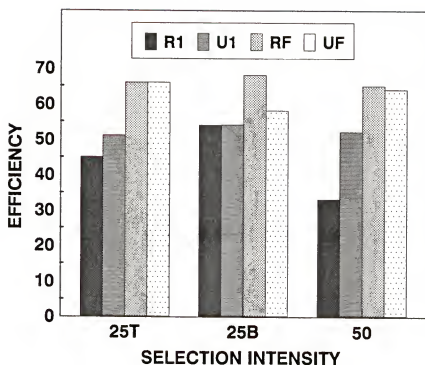Figure 3.1. Comparison of selection efficiency for fusiform rust using family averages from a single greenhouse test and field test for unimproved (U1 and UF) and improved (R1 and RF) populations of slash pine families. 25T, 25B are selection of the 25% top and bottom of the population, respectively. R1 and U1 are for the index developed using greenhouse information and RF and UF apply to field results.

percent of rust varies with year of planting (i.e., environmental conditions, inoculum load etc.); years with especially low disease incidence are not uncommon (Griggs and Schmidt, 1977). There is no guarantee that a field test will have enough rust to allow discrimination among genotypes with different levels of rust resistance. For example, among the 45 test-age combinations available for this study, 14 test-age combinations did not have enough rust (i.e., less than 20% rust) to allow discrimination among families with different levels of resistance. Even after eliminating these tests, the average for the remaining 31 test-age combination varied from 20 to 90% rust.

Other shortcomings of field tests are the length of time required for the test (i.e., 3 to 10 years), cost, and possible bias on growth trait evaluation caused by the disease (Hodge and White, 1986). The bias on growth trait expression has practical implications for breeding programs such as the CFGRP for slash pine, in which cooperators have different breeding priorities. Especially for cooperators with few timberlands in high hazard areas, the potential bias of growth rankings caused by high disease incidence in progeny tests reduces possible genetic gain in growth. The greenhouse tests at the RSC, on the other hand, are very repeatable, offer all adequate conditions for fungus infection and disease development (i.e., temperature, high relative humidity, inoculum load, fertilization and irrigation, etc.), are relatively inexpensive and the final results can be obtained in 8 to 10 months.

Recommended Uses

Considering that the RSC technique is viable, and its efficiency relative to field testing has been determined, we now need to examine how best to incorporate this procedure into the slash pine breeding program. In brief, the slash pine breeding program has now completed its second generation of selection. Among the 886 selected genotypes, 404 are original first-generation selections (backward),

304 are the best individuals (forward selections) from the better of 2,700 full-sib families created by crossing first-generation selections, and 178 are untested infusions (White et al., 1989). The breeding values for rust resistance for the first generation selections were predicted from data of 4 to 20 field progeny tests, using best linear prediction (White and Hodge, 1988). There is no reason to test these backward selections at the RSC. For the forward selections, the breeding values were estimated as the mean parental breeding values plus an estimated incremental increase in breeding value from within family selection (Hodge et al., 1989).

Therefore, early tests at the RSC would be desirable for the infusions and maybe some of the 304 forward selections. These individuals may not be as resistant as the improved material used to develop the R1 and R2 indices, but they should be more resistant then the unimproved material used to develop U1 and U2 indices. Hence, it is most likely that the R1 index is more appropriate for these families. Considering that the R1 index was almost as efficient as field tests in selecting the bottom 25% of the population, a conservative approach would be to use the greenhouse test at the RSC to eliminate the worst 25% of these families. The 178 infusions and 304 forward selections could be screened in the RSC, and the breeding values predicted from R1 used to cull the bottom 25% from any further consideration. Then, the remaining families in the selected population could be tested in short-term field tests designed specially to assess rust resistance. These field tests should be planted at close spacing in high hazard areas in one or two different locations. The results obtained from the two testing techniques (greenhouse and short-term field tests) could then be combined in a selection index; thus allowing for better predictions of parental breeding values.

According to Talbert (1985), if early screening and field test criteria of selection are strongly correlated, such an approach (i.e., two stage selection) may lead to reduced total gain, compared to what should be expected from the normal

field tests alone. However, taking into account that normal field progeny tests require a long time for reliable results, the uncertainty of the results, and especially the bias effect of disease on growth traits, the overall gain from a two-stage selection may compensate the possible reduced gain for rust resistance. An alternative to this approach is to test all families once in both greenhouse and field tests, and combine the results into a single prediction of breeding values. A less conservative approach would use only the greenhouse test. If the overall goal is to select the top 25% of the families for breeding purposes or inclusion in an orchard, using the breeding values predicted from greenhouse tests may not result in selection of the best families, but above average families will be selected.

Research Perspective

It may be possible to increase the efficiency of greenhouse testing over field tests by 1) modifying the screening technique, or 2) using different procedures to construct the selection indices. One modification of the screening technique that may allow for better prediction is to reduce the inoculum density. Almost all the traits measured on fusiform rust-infected seedlings in the greenhouse are binary traits, and most of the time are on the extreme portion of the distribution where the ability to detect any variability among families is greatly reduced. By reducing the inoculum concentration it may be possible to spread out the data allowing for better discrimination among families and probably better predictions.

Different indices could also be developed by trying alternative traits, constructed by combining individual traits (for example, the way we created FAR, ENR, RES, and RSC), or simply by using squared functions of a given trait or the interaction between two traits. In terms of the procedures to develop the indices, some of the assumptions used to construct the selection indices may not be as adequate as we thought. For example, to construct c for each trait, we either used

an average correlation across all field and greenhouse tests, or we modeled the correlation as a function of the incidence of rust in the field. Other models including greenhouse information, such as the difference between the maximum and minimum value observed for the trait, the test means, etc. may improve the predictability of the model and the overall estimate of c. Also, we assumed that for each index type, V and c could be constructed by combining information of all groups and testing periods. Perhaps, by starting with estimating V and c and constructing indices for each group and testing period may result in different indices that are better than either U1 or R1.

Conclusions

Our results indicate that application of selection index to the early testing (i.e., greenhouse tests at the RSC) resulted in more efficient and easily interpreted predictions of parental breeding values for rust resistance in the field than the index currently used by the RSC. Two indices were adopted, one for the unimproved population with five traits (SYM, GAL, PAD, FAR, SHR), and another for improved populations with three traits (GAL, FAR, SHR). Even though the efficiency of selection by using the new indices was improved by using data from two greenhouse tests, the increase in efficiency was not large enough to justify testing the same material more than once.

With the drawbacks of both field and greenhouse testing in mind, we recommend that the breeding program for slash pine should be based on a combination of early testing in the greenhouse and short-term field testing designed especially to evaluate rust resistance. Results of both types of tests can then be combined into a single prediction of breeding values with a selection index or best linear prediction technique. In addition, we believe that both the greenhouse testing

technique as well the indices presented here can be improved.

CONCLUSIONS

This study was designed to develop predictions of field breeding values for fusiform rust resistance from early testing under greenhouse conditions. A total of 100 slash pine families plus six seedlots with different levels of field rust resistance were tested in three testing periods. The experiment was conducted at the RSC near Asheville, N.C, using a concentrated basidiospore suspension technique. The test was well-conducted by the personnel at the RSC, with no biological or logistical problems that reduced the quality of the results or invalidated the final conclusions.

The study was divided into three stages. First, we evaluated the effect of different testing periods on expression of 22 different fusiform rust symptoms on three standard slash pine seedlots with different levels of field rust resistance. Since ten out of the 22 traits have a binomial distribution, we also evaluated how the arcsin and logistic transformations of data affected the potential usefulness of the traits to discriminate among families with different levels of resistance. Three conclusions can be drawn for this stage: 1) Most traits consistently ranked the three seedlots with different levels of resistance across all testing periods, but trait incidence levels varied from test to test; therefore to compare families tested in different tests it will be imperative to adjust the family means for scale effects of the test in which they were tested; 2) Evaluation of a single gall per seedling yielded information as reliable as evaluation of all galls on a given seedling; and 3) Transformation of the binomial traits, especially use of logistic transformation, reduced interactions and should be adopted as part of the RSC Procedure.

The second stage deals with estimation of genetic parameters for the 16

most promising traits from stage one, using four independent groups of 25 slash pine families. All binomial traits were evaluated after logistic transformation of plot means. For 11 out of the 16 traits evaluated (i.e., SYM, GAL, PGA, PAD, ADR, THN, SHR, TPR, ENR, RES, and RSS), family x test interactions were of very little practical or biological importance. In general, for these traits, the estimated genetic parameters were reliable, had only minor differences among the four groups of families, and had relatively high family heritability (i.e., $h_f^2 > 0.70$). The other 5 traits, especially FR2, FAR and ROR, had a measurable amounts of family x test interaction and lower heritability, but were only weakly correlated with previous traits; so these traits may also be of value in a predictive index. Therefore, all 16 traits were considered as candidate traits to be included in an index to predict field breeding values.

Finally, the third stage deals with application of selection index theory to the prediction of field fusiform rust breeding values from measurements of one or more greenhouse traits. The results indicate that selection index theory applied to the greenhouse tests results in more efficient and easily interpretable predictions of parental breeding values for rust resistance in the field than the index currently used by the RSC. None of the indices developed using selection index were equally efficient for populations unimproved and improved for rust resistance. Hence, we selected two indices (U1 and U2) for use when testing unimproved populations with five traits (i.e., SYM, GAL, PAD, FAR, and SHR), and two more (R1 and R2) for use for improved populations with three traits (i.e., GAL, FAR, and SHR). U1 and R1 indices should be used when data are available from a single greenhouse test as currently implemented by the RSC, while U2 and R2 are applicable when all families have been screened in two testing periods. Equations generated assuming that data were available from two greenhouse tests resulted only in small increase in precision. Therefore, multiple testing is not warranted.

Selection based on the predicted breeding values for the unimproved population using U1, and for the improved population using R1 was less efficient than selection based on a single field progeny test of average quality. Nonetheless, the differences in selection efficiency were not large enough to rule out greenhouse testing as an alternative or additional testing technique for fusiform rust. Also, if we take into account that normal field progeny tests are 1) relatively more expensive than greenhouse testing, 2) there is no guaranty that enough disease will occur to allow differentiation among resistant and susceptible families, and 3) the fact that high incidence of disease in field progeny tests might bias growth trait evaluation, the value of using greenhouse tests to select for fusiform rust may substantial. Therefore, we recommend that this early testing technique be used more intensively in the slash pine breeding program either as the only testing procedure for fusiform rust resistance or combined with short-term field tests in high hazard areas designed especially to evaluate rust.

In addition, both the greenhouse testing procedure, as well as the index presented here, could be improved and this should not be ignored. Modifications of the greenhouse screening technique that may allow better prediction are 1) reduction of inoculum concentration, and 2) use of alternative traits created by combining individual traits (for example the way we created FAR) or simply by using the squared of a given trait or interaction between two traits. In terms of procedures to develop the indices, it is possible that better estimation of c can be obtained by improving the model used to predict family mean correlation between field and greenhouse results. Such an improvement may be possible by including in the predictive model other variables beside incidence of rust on the field, as for example, the difference between the maximum and minimum value observed for the greenhouse trait.

APPENDIX 1
AECIOSPORE COLLECTION AREAS

Code[a]	Area Name	Area Counties
S-5	Mobile, AL	Mobile, Baldwin, and Escambia, AL Escambia and Santa Rosa, FL
S-6	Bainbridge, GA	Miller, Baker, Michell, Seminole, Decatur, and Grady
S-8	Perry, FL	Dixie and Taylor
S-9	Jacksonville, FL	Nassau, Duval, Clay, St. Johns
S-10	Savannah, GA	Effingham, Chatham, Bryan, Liberty, Evans, and Tattnall

[a] All sources of inoculum were mixed prior to use.

TEST SCHEDULE AT THE RSC FOR EACH PERIOD AND GROUP OF 25 SLASH PINE FAMILIES

	Testing	Operations			
Group[a]	Period	Planting	Transplant	Inoculation	Reading
3	1	Nov 9/87	Nov 30/87	Jan 5-6/88	Jun 20-24/88
1	1	Nov 16/87	Dec 7/87	Jan 12-13/88	Jun 27-30/88
2	1	Nov 23/87	Dec 14/87	Jan 20-21/88	Jul 5-8/88
4	1	Nov 30/87	Dec 21/87	Jan 26-27/88	Jul 11-15/88
2	2	Apr 11/88	May 2/88	Jun 7-8/88	Nov 21-25/88
4	2	Apr 18/88	May 9/88	Jun 14-15/88	Nov 28-30/88
3	2	Apr 25/88	May 16/88	Jun 21-22/88	Dec 5-9/88
1	2	May 2/88	May 23/88	Jun 28-29/88	Dec 12-16/88
1	3	Jul 18/88	Aug 8/88	Sep 13-14/88	Feb 27-Mar3/89
3	3	Jul 25/88	Aug 15/88	Sep 20-21/88	Mar 6-10/89
4	3	Aug 1/88	Aug 22/88	Sep 27-28/88	Mar 13-17/89
2	3	aug 8/88	Aug 29/88	Oct 4-5/88	Mar 20-24/89

[a] For each group and testing period, in addition to the 25 families of the group, 6 bulked seedlots with different levels of resistance were inoculated.

APPENDIX 3
FIELD TESTS LOCATIONS AND OVERALL TEST MEANS FOR RUST INFECTION AT AGES 3, 5, AND 10

Groups	ID[a]	Location	Mean Rust (%)		
			3[b]	5[b]	10[b]
1	33B	Webster Co, GA	57.6	65.2	-
1	34B	Taylor Co, FL	39.6	45.1	-
1	35B	Bulloch Co, GA	-	23.3	-
1	2-6	Lafayette Co, FL	-	44.9	-
2	33C	Webster Co, GA	25.4	73.5	-
2	35C	Bulloch Co, GA	-	37.1	-
2	10-9	Okaloosa Co, FL	-	25.7	21.6
2	10-10	Okaloosa Co, FL	-	58.8	81.7
3	32A	Escambia Co, AL	26.9	30.8	-
3	33A	Webster Co, GA	85.3	90.5	-
3	34A	Taylor Co, FL	31.8	42.2	-
3	35A	Bulloch Co, GA	50.5	55.6	-
4	33.1	Webster Co, GA	55.1	63.1	-
4	34.2	Taylor Co, FL	25.5	27.9	-
4	1-25	Wayne Co, GA	-	-	38.4
4	1-26	Wayne Co, GA	-	20.3	37.3
4	1-27	Wayne Co, GA	-	26.9	45.1
4	1-28	Wayne Co, GA	-	24.9	44.5

[a] ID refers to the CFGRP identification.
[b] 3, 5, and 10 are ages in which the tests were measured.

FAMILY MEAN CORRELATIONS BETWEEN GREENHOUSE TRAITS AND INCIDENCE OF RUST FOR THE FIELD TESTS AND BETWEEN GREENHOUSE TRAITS AND FIELD PREDICTED R50'S.

Traits	All Groups		Unimproved		Improved	
	$r_{f:G,F}$[a]	$r_{f:G,R50}$[b]	$r_{f:G,F}$[a]	$r_{f:G,R50}$[b]	$r_{f:G,F}$[a]	$r_{f:G,R50}$[b]
HEA	-0.230[c]	-0.230	-0.228[c]	-0.266	-0.136	-0.121
SYM	-0.358[c]	-0.337	-0.387[c]	-0.412	-0.150	-0.113
GAL	0.351[c]	0.364	0.353[c]	0.433	0.180	0.158
PGA	0.354[c]	0.342	0.381[c]	0.422	0.144	0.100
PAD	0.244	0.317	0.267	0.331	0.174	0.274
ADR	0.174	0.218	0.188	0.229	0.131	0.189
FAR	0.202	0.323	0.187	0.290	0.247	0.423
FR2	0.101	0.196	0.062	0.131	0.217	0.390
ROR	-0.021	-0.008	-0.056	-0.037	0.085	0.078
SHR	-0.311	-0.392	-0.315	-0.382	-0.299	-0.422
THN	-0.281	-0.379	-0.287	-0.369	-0.264	-0.407
TPR	0.263	0.320	0.295	0.328	0.168	0.297
ENR	-0.344[c]	-0.352	-0.353[c]	-0.422	-0.168	-0.140
RES	-0.243	-0.338	-0.245	-0.313	-0.236	-0.410
RSS	-0.374[c]	-0.360	-0.403[c]	-0.436	-0.161	-0.132

[a] Average family mean correlation between greenhouse traits and incidence of rust for the field tests.

[b] Average family mean correlation between greenhouse traits and predicted breeding values from field results.

[c] Family mean correlation predicted using models form Table 3.4.

PREDICTIONS FROM U1, RSC INDEX AND THE FIELD PREDICTED
BREEDING VALUES (R50'S) FOR THE UNIMPROVED POPULATION FOR
THE DIFFERENT TESTING PERIODS

Group	UFNUM[a]	R50	RSC$_1$[b]	U1$_1$[c]	RSC$_2$[b]	U1$_2$[c]	RSC$_3$[b]	U1$_3$[c]
1	156	35.5	155.6	50.6	99.5	56.6	127.2	49.0
1	556	22.7	224.1	38.8	154.5	42.7	187.1	44.1
1	656	10.6	220.5	39.6	162.2	37.8	141.5	38.4
1	756	24.6	214.9	25.5	279.6	20.2	251.6	23.8
1	1256	48.5	208.4	34.3	185.6	35.1	213.2	21.9
1	1356	61.8	128.3	58.8	154.7	55.2	151.2	39.5
1	1456	49.1	115.0	54.2	108.6	51.7	84.3	50.5
1	1758	49.7	85.2	67.2	63.1	64.9	62.6	73.3
1	1858	57.6	85.9	71.3	35.2	72.8	31.1	67.8
1	2358	53.6	205.9	42.1	176.1	37.6	105.1	53.4
1	2658	32.4	90.6	60.6	64.8	58.5	34.4	67.5
1	5355	35.2	105.9	50.6	67.2	61.5	85.7	57.7
1	21155	-2.6	253.9	25.6	253.0	39.9	227.1	20.3
1	24656	47.0	154.2	52.3	163.0	53.6	139.7	56.4
1	24756	56.6	107.3	56.2	113.5	55.3	87.2	60.4
1	24856	73.7	47.6	68.6	58.1	59.8	76.9	53.2
1	24956	56.2	94.2	54.5	130.0	50.2	65.3	54.9
1	25256	21.6	154.1	34.8	135.9	41.5	104.2	49.3
1	25456	57.9	62.5	71.8	57.6	69.1	59.8	63.9
1	29255	59.3	66.7	61.6	61.1	65.9	84.4	66.5
1	29355	19.2	135.4	50.5	142.8	40.5	124.3	42.4
1	29455	30.5	166.6	47.0	163.8	44.1	150.5	49.4
1	35756	8.2	175.9	33.5	212.9	35.5	201.4	46.1
2	2260	41.1	129.0	63.9	133.8	51.7	66.2	50.2
2	2760	52.1	95.3	59.8	178.4	52.9	41.5	47.7
2	3160	62.9	58.3	56.1	151.9	57.7	59.7	67.2
2	4061	1.8	152.9	40.3	92.7	52.3	71.0	43.6
2	4961	34.2	93.4	55.3	139.4	42.0	42.2	52.5
2	10856	73.3	181.6	51.9	143.8	59.1	117.4	58.5
2	12156	75.1	109.1	57.0	107.6	58.8	70.7	54.0
2	16958	38.8	242.6	18.4	169.4	33.6	135.5	25.3
2	18957	16.5	308.4	16.2	261.8	11.7	289.8	20.3
2	19157	39.2	88.1	58.8	139.6	50.6	38.9	54.8
2	20357	41.8	123.6	52.4	48.0	64.1	22.9	61.6

Group	UFNUM[a]	R50	RSC$_1$[b]	U1$_1$[c]	RSC$_2$[b]	U1$_2$[c]	RSC$_3$[b]	U1$_3$[c]
2	25455	72.5	136.3	62.4	150.4	56.6	84.8	44.4
2	26155	28.5	134.7	52.1	159.3	42.1	41.3	48.1
2	26255	42.9	61.7	50.5	105.6	66.0	18.5	56.8
2	26455	53.0	86.6	55.8	129.9	62.7	77.1	53.8
2	29656	64.6	129.3	50.0	168.8	35.4	82.4	31.4
2	29756	51.5	136.2	54.8	152.6	60.6	44.1	58.1
2	30356	44.3	199.0	31.0	129.5	49.2	71.7	46.0
2	30656	65.3	110.0	58.0	104.6	63.7	47.8	59.5
2	30856	42.0	149.9	31.1	154.7	32.6	203.3	23.3
2	30956	93.3	147.3	51.4	132.7	46.9	58.3	62.0
2	31156	61.1	121.6	63.5	74.7	80.6	51.1	73.3
2	31556	50.1	133.2	52.1	155.7	52.8	132.5	47.7
2	31756	29.1	116.0	55.0	188.8	33.5	94.5	48.9
2	31856	52.3	149.8	52.5	206.9	32.8	80.7	61.0
3	M 14	1.7	299.1	14.7	254.1	19.2	307.7	18.9
3	M 118	56.6	99.2	64.0	84.3	55.4	159.0	56.9
3	M 121	41.6	115.7	56.7	180.0	51.2	125.8	55.7
3	M 122	54.0	118.6	55.9	165.7	38.4	118.0	50.5
3	M 123	42.4	161.4	37.8	157.0	34.7	196.9	28.3
3	M 124	51.0	232.1	40.8	157.8	46.5	195.1	45.4
3	M 211	59.0	113.3	58.1	111.6	57.4	118.3	54.0
3	M 213	81.5	102.4	65.8	137.9	65.1	94.2	67.7
3	M 404	63.9	190.3	42.4	142.4	49.3	120.8	44.0
3	M 502	46.0	84.8	46.4	119.8	45.7	92.9	49.2
3	M 503	66.8	97.4	55.4	110.2	57.3	90.4	52.0
3	M 604	42.5	36.6	64.0	66.8	70.0	40.5	61.8
3	M 720	60.4	160.1	45.9	201.9	39.7	121.2	47.2
3	M 727	86.6	70.0	62.8	124.2	64.1	87.4	58.5
3	M 728	52.5	199.1	30.8	244.5	36.0	232.0	36.5
3	M 738	63.1	112.5	57.0	140.3	53.4	80.2	52.0
3	M 814	55.9	188.1	37.7	125.3	51.1	143.8	47.4
3	M 821	51.7	144.5	49.2	120.8	53.7	119.3	46.4
3	M 828	58.6	140.5	49.2	117.1	45.6	96.0	48.2
3	M 831	64.6	138.0	58.6	116.4	61.9	105.7	60.6
3	M 832	42.3	165.1	58.2	98.1	55.0	94.6	54.8
3	M 835	13.8	137.7	42.0	125.4	47.9	149.4	55.0
3	M 908	38.2	132.1	56.6	123.9	51.4	132.4	59.0

[a] Family numbers as designated by the Cooperative Forest Genetics Research Program.
[b] RSC$_1$, RSC$_2$, RSC$_3$ are predictions obtained using the RSC index and data from testing periods 1, 2, and 3, respectively.
[c] U1$_1$, U1$_2$, U1$_3$ are predictions obtained using the selection index developed for the unimproved population and data from testing periods 1, 2, and 3, respectively.

PREDICTIONS FROM R1, RSC INDEX AND THE FIELD PREDICTED
BREEDING VALUES (R50'S) FOR THE IMPROVED POPULATION FOR
THE DIFFERENT TESTING PERIODS

Group	UFNUM[a]	R50	RSC$_1$[b]	R1$_1$[c]	RSC$_2$[b]	R1$_2$[c]	RSC$_3$[b]	R1$_3$[c]
4	C 66	67.5	110.0	44.3	135.3	64.1	111.6	49.5
4	C 67	27.3	95.2	42.1	192.6	32.8	137.2	35.5
4	C 73	26.8	186.5	21.5	192.5	25.7	101.9	34.8
4	C 102	-7.8	200.4	18.3	271.6	15.5	157.5	19.4
4	C 108	44.6	144.7	40.1	168.0	33.2	157.6	29.9
4	C 109	22.5	121.3	26.4	66.4	39.1	130.8	40.8
4	C 112	36.0	175.3	29.2	238.8	30.6	161.9	28.9
4	C 115	34.3	205.2	34.5	204.8	45.5	155.3	30.6
4	C 126	63.9	66.6	46.1	143.6	43.0	54.1	46.9
4	C 127	49.3	101.8	37.7	132.4	37.7	103.9	44.4
4	C 128	35.0	103.9	46.9	165.4	43.0	90.3	52.9
4	C 129	27.1	250.1	25.6	261.3	30.7	150.1	33.6
4	C 137	37.7	188.7	39.2	199.9	33.6	158.0	34.1
4	C 138	30.3	119.8	39.7	164.2	26.7	104.7	33.7
4	C 139	14.9	96.3	40.6	153.4	39.2	108.4	40.4
4	C 145	26.4	95.1	45.0	132.8	33.4	94.2	36.2
4	C 147	33.1	85.4	46.3	124.2	43.0	84.6	41.8
4	C 161	-4.5	63.5	42.0	130.0	42.5	75.2	44.5
4	C 171	32.1	113.6	46.3	169.3	44.5	140.7	41.1
4	C 180	28.9	149.9	25.9	120.3	33.1	94.7	30.1
4	C 200	16.2	178.3	25.3	147.2	36.3	225.2	18.6
4	C 201	22.5	180.9	29.8	288.7	24.9	234.7	17.7
4	C 212	55.9	80.9	52.3	140.9	39.2	86.1	48.5
4	C 221	37.7	148.6	26.8	214.1	24.2	94.1	30.7
4	C 225	35.0	214.6	28.1	192.6	38.6	79.6	36.0

[a] Family numbers as designated by the Cooperative Forest Genetics Research Program.
[b] RSC$_1$, RSC$_2$, RSC$_3$ are predictions obtained using the RSC index and data from testing periods 1, 2, and 3, respectively.
[c] R1$_1$, R1$_2$, R1$_3$ are predictions obtained using the selection index developed for the improved population and data from testing periods 1, 2, and 3, respectively.

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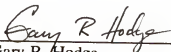
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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Timothy L. White, Chairman
Associate Professor of Forest Resources and Conservation

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Gary R. Hodge
Assistant Research Scientist, Forest Resources and Conservation

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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Professor of Forest Resources and Conservation

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Donald L. Rockwood
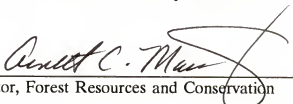Professor of Forest Resources and Conservation

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Ramon C. Littell
Professor of Statistics

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the School of Forest Resources and Conservation in the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy

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